



(12) **DEMANDE DE BREVET CANADIEN**
CANADIAN PATENT APPLICATION
(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2017/05/04
(87) Date publication PCT/PCT Publication Date: 2017/11/09
(85) Entrée phase nationale/National Entry: 2018/10/04
(86) N° demande PCT/PCT Application No.: EP 2017/060621
(87) N° publication PCT/PCT Publication No.: 2017/191239
(30) Priorités/Priorities: 2016/05/04 (EP16168448.5);
2016/11/18 (EP16199502.2)

(51) Cl.Int./Int.Cl. *C12N 9/88* (2006.01),
C12P 5/02 (2006.01)
(71) Demandeur/Applicant:
GLOBAL BIOENERGIES, FR
(72) Inventeurs/Inventors:
STRICHER, FRANCOIS, FR;
VILLIERS, BENOIT, FR
(74) Agent: LAVERY, DE BILLY, LLP

(54) Titre : VARIANTES DE LA DECARBOXYLASE DE L'ACIDE 3-METHYLCROTONIQUE (MDC)
(54) Title: 3-METHYLCROTONIC ACID DECARBOXYLASE (MDC) VARIANTS

(57) **Abrégé/Abstract:**

Described are 3-methylcrotonic acid decarboxylase (MDC) variants showing improved activity in converting 3-methylcrotonic acid into isobutene as well methods for the production of isobutene using such enzyme variants.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(10) International Publication Number
WO 2017/191239 A1

(43) International Publication Date
09 November 2017 (09.11.2017)

(51) International Patent Classification:

C12N 9/88 (2006.01) *C12P 5/02* (2006.01)

(21) International Application Number:

PCT/EP2017/060621

(22) International Filing Date:

04 May 2017 (04.05.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

16168448.5 04 May 2016 (04.05.2016) EP
16199502.2 18 November 2016 (18.11.2016) EP

(71) Applicant: **GLOBAL BIOENERGIES** [FR/FR]; 5 rue
Henri Desbruères, 91000 Evry (FR).

(72) Inventors: **STRICHER, François**; 20 rue Berlioz, 67202
Wolfisheim (FR). **VILLIERS, Benoît**; 19 rue de Flore,
94140 Alfortville (FR).

(74) Agent: **VOSSIUS & PARTNER**; Siebertstraße 3, 81675
München (DE).

(81) Designated States (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR,
KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR,
TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every
kind of regional protection available*): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: 3-METHYLCROTONIC ACID DECARBOXYLASE (MDC) VARIANTS

(57) Abstract: Described are 3-methylcrotonic acid decarboxylase (MDC) variants showing improved activity in converting 3-methylcrotonic acid into isobutene as well methods for the production of isobutene using such enzyme variants.

WO 2017/191239 A1

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 177

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 177

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

3-Methylcrotonic acid decarboxylase (MDC) variants

Described are 3-methylcrotonic acid decarboxylase (MDC) variants showing an improved activity in converting 3-methylcrotonic acid into isobutene as well as methods for the production of isobutene using such enzyme variants.

A large number of chemical compounds are currently derived from petrochemicals. Alkenes (such as ethylene, propylene, the different butenes, or else the pentenes, for example) are used in the plastics industry, for example for producing polypropylene or polyethylene, and in other areas of the chemical industry and that of fuels.

Butylene exists in four forms, one of which, isobutene (also referred to as isobutylene), enters into the composition of methyl-tert-butyl-ether (MTBE), an anti-knock additive for automobile fuel. Isobutene can also be used to produce isooctene, which in turn can be reduced to isooctane (2,2,4-trimethylpentane); the very high octane rating of isooctane makes it the best fuel for so-called "gasoline" engines. Alkenes such as isobutene are currently produced by catalytic cracking of petroleum products (or by a derivative of the Fischer-Tropsch process in the case of hexene, from coal or gas). The production costs are therefore tightly linked to the price of oil. Moreover, catalytic cracking is sometimes associated with considerable technical difficulties which increase process complexity and production costs.

The production by a biological pathway of alkenes such as isobutene is called for in the context of a sustainable industrial operation in harmony with geochemical cycles. The first generation of biofuels consisted in the fermentative production of ethanol, as fermentation and distillation processes already existed in the food processing industry. The production of second generation biofuels is in an exploratory phase, encompassing in particular the production of long chain alcohols (butanol and pentanol), terpenes, linear alkanes and fatty acids. Two recent reviews provide a general overview of research in this field: Ladygina et al. (Process Biochemistry 41 (2006), 1001) and Wackett (Current Opinions in Chemical Biology 21 (2008), 187).

Different routes for the enzymatic generation of isobutene have previously been described; see, e.g., Fujii et al. (Appl. Environ. Microbiol. 54 (1988), 583); Gogerty et al. (Appl. Environ. Microbiol. 76 (2010), 8004-8010) and van Leeuwen et al. (Appl. Microbiol. Biotechnol. 93 (2012), 1377-1387) and WO2010/001078.

In addition to these routes, there are also alternative routes for the provision of isobutene utilizing the enzymatic conversion of 3-methylcrotonic acid into isobutene

by a decarboxylation reaction. A decarboxylation is a chemical reaction that removes a carboxyl group and releases carbon dioxide (CO₂).

The decarboxylation of 3-methylcrotonic acid has already been suggested in US-A1-2009/0092975 while there is no experimental evidence for this conversion. In US-A1-2009/0092975, a nucleic acid sequence called PAD1 derived from *Saccharomyces cerevisiae* is described and is disclosed to encode a decarboxylation enzyme. This enzyme is suggested to be useful as a selectable marker in a recombinant organism while it is described that a "weak acid" may be used as the selecting agent. 3-methylcrotonic acid is mentioned, among many others, as a potential "weak acid".

However, it was only later found that the above PAD1, in reality, does not provide for the decarboxylase activity.

In fact, the bacterial *ubiD* and *ubiX* or the homologous eukaryotic *fdc1* and *pad1* genes have been implicated in the non-oxidative reversible decarboxylation. The combined action of phenylacrylic acid decarboxylase (PAD) and ferulic acid decarboxylase (FDC) is considered to be essential for the decarboxylation of phenylacrylic acid in *Saccharomyces cerevisiae* (J. Biosci. Bioeng. 109, (2010), 564-569; AMB Express, 5:12 (2015) 1-5; ACS Chem. Biol. 10 (2015), 1137-1144).

Recently, the above enzyme family described as phenylacrylic acid decarboxylase (PAD) was characterized as an FMN prenyl-transferase and no longer as a decarboxylase. It has been shown that Fdc1 (but not PAD) is solely responsible for the reversible decarboxylase activity and that it requires a new type of cofactor, namely a prenylated flavin synthesized by the associated UbiX (or Pad1) protein. Thus, the real enzymatic activity of this PAD enzyme has been identified as the transformation of a flavin mononucleotide (FMN) cofactor with a prenyl moiety (from di-methyl-allyl-phosphate or pyrophosphate called DMAP or DMAPP). This reaction is shown in **Figure 1A**.

Accordingly, in contrast to the prior art's belief, the real decarboxylase is the Ferulic Acid Decarboxylase (FDC) in association with the modified FMN (prenylated-FMN). This reaction is shown in **Figure 1B**. This mechanism of the Ferulic Acid Decarboxylase (FDC) in association with the modified FMN (prenylated-FMN) (the latter provided by the PAD enzyme) was recently described and involves a surprising enzymatic mechanism, i.e., an α,β -unsaturated acid decarboxylation via a 1,3-dipolar cyclo-addition. Moreover, the structure of this FDC decarboxylase has recently been elucidated (Nature 522 (2015), 497-501; Nature, 522 (2015), 502-505; Appl. Environ. Microbiol. 81 (2015), 4216- 4223).

Although the above means and methods allow to produce isobutene from 2-methylcrotonic acid, there is still a need for improvements, in particular as regards a

further increase in efficiency of the process so as to make it more suitable for industrial purposes.

The present application addresses this need by providing the embodiments as defined in the claims.

In a first aspect, the present invention provides a variant of a 3-methylcrotonic acid decarboxylase (MDC) showing an improved activity in converting 3-methylcrotonic acid into isobutene over the corresponding MDC from which it is derived.

An improved enzyme variant or an enzyme variant capable of catalyzing a reaction with increased activity is defined as an enzyme variant which differs from the wildtype enzyme and which catalyzes the conversion of 3-methylcrotonic acid into isobutene so that the specific activity of the enzyme variant is higher than the specific activity of the wildtype enzyme for at least one given concentration of a 3-methylcrotonic acid (preferably any 3-methylcrotonic acid higher than 0 M and up to 1 M). A specific activity is defined as the number of moles of substrate converted to moles of product by unit of time by mole of enzyme. K_{cat} (turnover number) is the specific activity at saturating concentration of substrate.

In particular, in accordance with this first aspect, the present invention provides enzymes which are capable of converting 3-methylcrotonic acid into isobutene with a turnover rate of at least $1 \times 10^{-3} \text{ s}^{-1}$ of 3-methylcrotonic acid into isobutene. Such enzymes can be provided by effecting mutations at specific positions in an 3-methylcrotonic acid decarboxylase (MDC) and the variants obtained by effecting such mutations show an improved activity in catalyzing the conversion of 3-methylcrotonic acid into isobutene. In a preferred embodiment, the enzyme is capable of converting 3-methylcrotonic acid into isobutene with a turnover rate of at least $2 \times 10^{-3} \text{ s}^{-1}$ of 3-methylcrotonic acid into isobutene and in a particularly preferred embodiment of at least $4 \times 10^{-3} \text{ s}^{-1}$. In a most preferred embodiment, the enzyme has a turnover rate of at least $10 \times 10^{-3} \text{ s}^{-1}$ or at least 1 s^{-1} , or at least 10 s^{-1} and even more preferably of at least 100 s^{-1} of 3-methylcrotonic acid into isobutene. The corresponding wild-type enzyme has a turnover rate of about $1 \times 10^{-3} \text{ s}^{-1}$ of 3-methylcrotonic acid into isobutene.

In the context of the present invention, an "improved activity" means that the activity of the enzyme in question is at least 10%, preferably at least 20%, more preferably at least 30% or 50%, even more preferably at least 70% or 80% and particularly preferred at least 90% or 100% higher than that of the enzyme from which the

variant is derived, preferably higher than that of the enzyme represented by SEQ ID NO:1. In even more preferred embodiments the improved activity may be at least 150%, at least 200%, at least 300%, at least 750% or at least 1000% higher than that of the corresponding enzyme from which the variant is derived, preferably higher than that of the enzyme represented by SEQ ID NO:1. In a particularly preferred embodiment, the activity is measured by using an assay with purified enzyme and chemically synthesized substrates, as described below. The improved activity of a variant can be measured as a higher isobutene production in a given time under defined conditions, compared with the parent enzyme. This improved activity can result from a higher turnover number, e.g. a higher k_{cat} value. It can also result from a lower K_m value. It can also result from a higher k_{cat}/K_m value. Finally, it can result from a higher solubility, or stability of the enzyme. The degree of improvement can be measured as the improvement in isobutene production. The degree of improvement can also be measured in terms of k_{cat} improvement, of k_{cat}/K_m improvement, or in terms of K_m decrease, in terms of soluble protein production or in terms of protein stability.

In another embodiment, the enzyme variants which the present invention provides are capable of converting 3-methylcrotonic acid into isobutene with an activity which is at least 1.25 times as high compared to the turnover rate of the corresponding wild type enzyme having the amino acid sequence as shown in SEQ ID NO:1. In a more preferred embodiment, the enzyme variants which are capable of converting 3-methylcrotonic acid into isobutene have a turnover rate (i.e., a k_{cat} -value) which is at least 2 times, at least 3 times, at least 5 times or even at least 10 times as high compared to the turnover rate of the corresponding wild type enzyme having the amino acid sequence as shown in SEQ ID NO:1. In even more preferred embodiments, the turnover rate is at least 100 times or even at least 500 times as high compared to that of the corresponding wild type enzyme having the amino acid sequence as shown in SEQ ID NO:1.

Such enzyme variants are obtained by effecting mutations at specific positions in the amino acid sequence of an MDC and the variants obtained by effecting such mutations show an improved activity in catalyzing the conversion of 3-methylcrotonic acid into isobutene. The activity of an enzyme capable of converting 3-methylcrotonic acid into isobutene may be determined by methods known to the person skilled in the art. In one embodiment, this activity is determined as described in the Examples appended hereto. In a particular embodiment this activity can be measured by incubating the enzyme, preferably a cell lysate containing the overexpressed

recombinant protein, in vitro. Alternatively, a purified enzyme can be used or an in vivo assay.

More specifically, the activity of the MDC variants for the conversion of 3-methylcrotonic acid into isobutene can be assessed by an enzymatic in vitro assay based on purified proteins and on the detection of isobutene by gas chromatography. The turnover rate of the enzyme to be assessed may be examined as outlined in the following: Michaelis-Menten k_{cat} and K_m steady state kinetic constants for the reaction of conversion of 3-methylcrotonic acid into isobutene may be determined using the following protocol:

The enzymatic assay for quantifying the conversion of 3-methylcrotonic acid into isobutene is carried out in a 2 ml glass vial at 30°C in a 50mM potassium phosphate pH 7.5 buffer; 20 mM NaCl, 3 mM $MgCl_2$, 5mM DTT, 0.5 mg/ml of a purified enzyme of the MDC variant to be tested, 100 μ l of a lysate containing a FMN prenyltransferase (i.e., a Flavin prenyltransferase UbiX protein from *E. coli* expressed and prepared as outlined further below) as well as different concentrations of the substrate 3-methylcrotonic acid ranging from 0 to 128 mM. A control without an MDC enzyme is performed in parallel. After 60 minutes, the reaction is stopped by incubating at 80°C for 2 min. The rate of isobutene production is quantified by gas chromatography as follows.

The isobutene formed in the reaction headspace is analysed by gas chromatography (GC) equipped with a flame ionization detector (FID). For the GC headspace analysis, one ml of the headspace gas is separated in a Bruker GC-450 system equipped with a GS-alumina column (30 m x 0.53 mm) (Agilent) using isothermal mode at 130°C. Nitrogen is used as carrier gas with a flow rate of 6 ml/min. The enzymatic reaction product is identified by comparison with an isobutene standard. Under these GC conditions, the retention time of isobutene is 2.42 min. From the rate of isobutene production, and using the Michaelis-Menten approximation, the enzyme catalytic efficiency can then be computed. The production rates of isobutene (mole of PV/mole enzyme/sec) are plotted as a function of the concentration of 3-methylcrotonic acid and the curve is fitted using the Michaelis Menten equation ($V=(V_{max}*(substrate))/(K_m+(substrate))$) to extract the k_{cat} (s^{-1}) and the K_m values (mM).

The MDC variant to be tested can be provided according to the following protocol: The MDC to be tested is subcloned into the pETDuet™-1 co-expression vector. The vector contains a stretch of 6 histidine codons after the methionine initiation codon of the ferulic acid decarboxylases in order to provide an affinity tag for purification.

Competent *E. coli* BL21 (DE3) cells (Novagen) are transformed with this vector according to standard heat shock procedures and plated out onto LB-agar plates supplemented with the appropriate antibiotic. Cells are grown overnight at 30°C until individual colonies reach the desired size. A single colony is then picked and individually transferred into 5 ml of liquid LB medium supplemented with the appropriate antibiotic. Cell growth is carried out with shaking for 16 hours at 30°C. The LB culture of the transformed cells is used to inoculate a culture using ZYM-5052 auto-induction medium (Studier FW, Prot. Exp. Pur. 41, (2005), 207-234) and the culture is grown with shaking (160 rpm) at 30°C during 24 h. The cells are collected by centrifugation at 4°C, 10,000 rpm for 20 min and the pellets are frozen and stored at -80°C. The pellets containing the overexpressed protein of a 500 ml of cultured cells is thawed on ice and resuspended in 15 ml of 50 mM potassium phosphate buffer containing 200 mM NaCl, 10 mM MgCl₂, 10 mM imidazole and 1 mM DTT. Twenty microliters of lysonase (Novagen) is added and the cells are incubated for 10 minutes at room temperature and then returned to ice for 20 minutes. Cell lysis is then completed by sonication for 2 x 15 seconds.

The bacterial extracts are then clarified by centrifugation at 4°C, 4000 rpm for 40 min. The clarified bacterial lysates are loaded onto a PROTINO-2000 Ni-TED column (Macherey-Nagel) allowing adsorption of 6-His tagged proteins. Columns are washed and the enzymes of interest are eluted with 6 ml of 50 mM potassium phosphate buffer containing 250 mM imidazole. Eluates are then concentrated, desalted on a Amicon Ultra-4 10 kDa filter unit (Millipore) and enzymes are resuspended in 50 mM potassium phosphate buffer containing 1 mM DTT and 20 mM NaCl. Protein concentrations are determined by direct UV 280 nm measurement on a NanoDrop 1000 spectrophotometer (Thermo Scientific) or by a Bradford assay (BioRad).

Correspondingly, the cDNA of a Flavin prenyltransferase UbiX protein from *E. coli* is cloned and recombinantly expressed, purified and quantified.

As described in the above enzymatic in vitro assay for determining the activity of the MDC variants of the present invention, UbiX does not necessarily have to be provided in a recombinantly expressed and subsequently purified manner. Therefore, UbiX may alternatively also be provided in the form of a UbiX-containing cell lysate without purifying it as described in the following.

The Flavin prenyltransferase UbiX protein from *E. coli* is cloned in the vector pCAN. The Flavin prenyltransferase UbiX protein from *E. coli* was purchased from NAIST (Nara Institute of Science and Technology, Japan, ASKA collection).

Competent *E. coli* BL21 (DE3) cells (Novagen) are transformed with this vector according to standard heat shock procedures and plated out onto LB-agar plates

supplemented with the appropriate antibiotic. Cells are grown overnight at 30°C until individual colonies reach the desired size. A single colony is then picked and individually transferred into 5 ml of liquid LB medium supplemented with the appropriate antibiotic. Cell growth is carried out with shaking for 16 hours at 30°C. The LB culture of the transformed cells is used to inoculate a ZYM-5052 auto-induction medium (Studier FW, Prot. Exp. Pur. 41, (2005), 207-234) and the culture is grown with shaking (160 rpm) using at 30°C during 24 h. The cells are collected by centrifugation at 4°C, 10,000 rpm for 20 min and the pellets are stored at -80°C. Pellets from 500 ml of cultured cells are thawed on ice and resuspended in 15 ml of 50 mM potassium phosphate buffer containing 200 mM NaCl, 10 mM MgCl₂, 10 mM imidazole and 1 mM DTT. Twenty microliters of lysonase (Novagen) is added. Cells are incubated 10 minutes at room temperature and then returned to ice for 20 minutes. Cell lysis is completed by sonication for 2 x 15 seconds. The cellular lysate containing the UbiX protein is kept on ice.

Alternatively to the above *in vitro* assays, the activity of the MDC variants for the conversion of 3-methylcrotonic acid into isobutene can be assessed by an *in vivo* testing. This coupled *in vivo* assay is based on the use of a bacterial strain transformed with an expression vector that contains the coding sequences leading to the production of the MDC variant and the Flavin prenyltransferase UbiX protein from *E. coli* (SEQ ID NO:2). Thus, the MDC variant to be tested is subcloned into a pETDuet™-1 co-expression vector (Novagen) in addition to the cDNA of the Flavin prenyltransferase UbiX protein from *E. coli*.

The MDC variant of the present invention to be tested is used to catalyze the decarboxylation reaction of 3-methylcrotonic acid into isobutene while the Flavin prenyltransferase UbiX protein from *E. coli* provides the modified flavin cofactor. Thus, in the coupled *in vivo* assay, a bacterial strain is used which is transformed with the above expression vector.

The transformed strain is first plated out onto LB-agar plates supplemented with the appropriate antibiotic. Cells are then grown overnight at 30°C until individual colonies reach the desired size. Single colonies are then picked and individually transferred into either 50 or 500 µL of liquid LB medium supplemented with the appropriate antibiotic. Cell growth is carried out with shaking for 20 hours at 30°C. The LB cultures are used to inoculate 300 µL in 384 deepwell microplates or 1 mL in 96 deepwell microplates of auto-induction medium (Studier FW, Prat. Exp. Pur. 41, (2005), 207-234) supplemented with the appropriate antibiotic and grown in a shaking incubator set at 700rpm and 85% humidity for 24h at 30°C in order to produce the two types of recombinant enzymes. The cell pellet containing these overexpressed recombinant enzymes is then resuspended in 40 µL of minimum

medium (pH 7.5, Phosphate 100 mM, Glucose 10g.L⁻¹, MgSO₄ 1mM) supplemented with 10mM 3-methylcrotonic acid in 384 deepwell microplates or in 400 µL of minimum medium (pH 7.5, Phosphate 100 mM, Glucose 10g.L⁻¹, MgSO₄ 1mM) supplemented with 10 mM 3-methylcrotonic acid in 96 deepwell microplates and incubated for a further 2 or 4 hours in a shaking incubator at 37°C, 700 rpm. During this step, the MDC variant catalyses the decarboxylation of 3-methylcrotonic acid into isobutene. After 5 min inactivation at 80°C, the isobutene produced is quantified by gas chromatography as follows. 100 µL of headspace gases from each enzymatic reaction are injected in a Brucker GC-450 system equipped with a Flame Ionization Detector (FID). Compounds present in samples are separated by chromatography using a RTX-1 column at 100°C with a 1 mL/min constant flow of nitrogen as carrier gas. Upon injection, peak areas of isobutene are calculated.

By providing the above described enzyme variant, the present invention allows to dramatically increase the production efficiency of isobutene from 3-methylcrotonic acid.

The term “3-methylcrotonic acid decarboxylase (MDC)” refers to an enzyme which can catalyze the decarboxylation of 3-methylcrotonic acid into isobutene. A decarboxylation is a chemical reaction that removes a carboxyl group and releases carbon dioxide. This activity can be measured by methods known in the art and as described above. In a preferred embodiment, the MDC is a Ferulic Acid Decarboxylase (FDC) or is derived from such an enzyme. FDCs belong to the enzyme class EC 4.1.1.-. As mentioned above, it has originally been described that an FDC in association with a modified FMN (prenylated-FMN) is capable of catalyzing an α,β -unsaturated decarboxylation via a 1,3-dipolar cyclo-addition and, more specifically, capable of catalyzing the decarboxylation of 3-methylcrotonic acid into isobutene. Thus, in the context of the present invention, the term FDC relates to enzymes capable of catalyzing the decarboxylation of 3-methylcrotonic acid into isobutene, preferably when provided with a prenylated FMN.

FDC enzymes have, e.g., been described in *Saccharomyces cerevisiae*, *Enterobacter* sp., *Bacillus pumilus*, *Aspergillus niger* or *Candida dubliniensis*. Hence, in preferred embodiments, the FDC is derived from *Saccharomyces cerevisiae* (Uniprot accession number Q03034), *Enterobacter* sp. (Uniprot accession number V3P7U0), *Bacillus pumilus* (Uniprot accession number Q45361), *Aspergillus niger* (Uniprot accession number A2R0P7) or *Candida dubliniensis* (Uniprot accession number B9WJ66). In more preferred embodiments, the FDC is a 3-polyprenyl-4-

hydroxybenzoate decarboxylase (UbiD). 3-polyprenyl-4-hydroxybenzoate decarboxylases have, e.g., been described in *Hypocrea atroviridis*, *Sphaerulina musiva*, *Penicillium requeforti*, *Fusarium oxysporum* f. sp. *lycopersici*, *Saccharomyces kudriavzevii*, *Saccaromyces cerevisiae*, *Aspergillus parasiticus*, *Candida albicans*, *Grosmannia clavigera*, *Escherichia coli*, *Bacillus megaterium*, *Methanothermobacter* sp. CaT2 or *Mycobacterium chelonae* 1518. Hence, in more preferred embodiments, the FDC enzyme variant capable of catalyzing the decarboxylation of 3-methylcrotonic acid into isobutene is derived from a 3-polyprenyl-4-hydroxybenzoate decarboxylase (UbiD) from *Hypocrea atroviridis* (UniProt Accession number G9NLP8), *Sphaerulina musiva* (UniProt Accession number M3DF95), *Penicillium requeforti* (UniProt Accession number W6QKP7), *Fusarium oxysporum* f. sp. *lycopersici* (UniProt Accession number W9LTH3), *Saccharomyces kudriavzevii* (UniProt Accession number J8TRN5), *Saccaromyces cerevisiae*, *Aspergillus parasiticus*, *Candida albicans*, *Grosmannia clavigera*, *Escherichia coli* (Uniprot accession number P0AAB4), *Bacillus megaterium* (Uniprot accession number D5DTL4), *Methanothermobacter* sp. CaT2 (Uniprot accession number T2GKK5) or *Mycobacterium chelonae* 1518 (Uniprot accession number X8EX86). Preferably, the MDC is an enzyme which is associated with and/or depends on an FMN prenyl transferase. As mentioned above, the enzymatic conversion of 3-methylcrotonic acid into isobutene utilizing an FMN-dependent decarboxylase is preferably associated with an FMN prenyl transferase and relies on a reaction of two consecutive steps catalyzed by the two enzymes, i.e., the FMN-dependent decarboxylase (catalyzing the actual decarboxylation of 3-methylcrotonic acid into isobutene) with an associated FMN prenyl transferase which provides the modified flavin cofactor. The flavin cofactor may preferably be FMN or FAD. FMN (flavin mononucleotide; also termed riboflavin-5'-phosphate) is a biomolecule produced from riboflavin (vitamin B2) by the enzyme riboflavin kinase and functions as prosthetic group of various reactions. FAD (flavin adenine dinucleotide) is a redox cofactor, more specifically a prosthetic group, involved in several important reactions in metabolism. The FMN prenyl transferases which may be associated with the MDC variants of the present invention are described in more detail further below.

The present invention provides now improved variants of enzymes which are capable of converting 3-methylcrotonic acid into isobutene. The inventors used as a model enzyme the FDC of *Hypocrea atroviridis* shown in SEQ ID NO: 1 and could show that it is possible to provide variants of this enzyme which show increased activity with respect to the conversion of 3-methylcrotonic acid into isobutene.

The model enzyme, i.e., the FDC of *Hypocrea atroviridis*, as used by the inventors has the amino acid sequence as shown in SEQ ID NO:1.

In one preferred embodiment the variants of the present invention are characterized by the feature that they are derived from an MDC, more preferably from an MDC having the amino acid sequence shown in SEQ ID NO:1 or a highly related sequence (at least 60% identical) and in which mutations are effected at one or more of the above indicated positions and by the feature that they show the ability to convert 3-methylcrotonic acid into isobutene and that they can do this with an improved activity. In a preferred embodiment the variant according to the present invention is derived from a sequence which shows at least 70%, more preferably at least 80% sequence identity to SEQ ID NO:1 and in which one or more substitutions and/or deletions and/or insertions at the positions indicated herein have been effected.

However, the teaching of the present invention is not restricted to the MDC enzyme of *Hypocrea atroviridis* shown in SEQ ID NO: 1 which had been used as a model enzyme but can be extended to MDC enzymes from other organisms or to enzymes which are structurally related to SEQ ID NO:1 such as, e.g., truncated variants of the enzyme. Thus, the present invention also relates to variants of MDCs which are structurally related to the *Hypocrea atroviridis* sequence (SEQ ID NO: 1) and which show one or more substitutions and/or deletions and/or insertions at positions corresponding to any of the positions as indicated herein. The term "structurally related" refers to MDCs which show a sequence identity of at least n% to the sequence shown in SEQ ID NO: 1 with n being an integer between 60 and 100, preferably 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99. In a preferred embodiment the structurally related MDC stems from a fungus, more preferably from an organism of the division of Ascomyceta, even more preferably from an organism of the class of Sordariomycetes, the order of Hypocreales, the family of Hypocreaceae or the genus *Hypocrea*, most preferably of the genus *Hypocrea*.

Thus, in one embodiment, the variant of an MDC according to the present invention has or preferably is derived from a sequence which is at least n % identical to SEQ ID NO:1 with n being an integer between 60 and 100, preferably 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99, and it has (a) substitution(s) and/or (a) deletion and/or (an) insertion(s) at a position as indicated herein. When the sequences which are compared do not have the same length, the degree of identity either refers to the percentage of amino acid

residues in the shorter sequence which are identical to amino acid residues in the longer sequence or to the percentage of amino acid residues in the longer sequence which are identical to amino acid residues in the shorter sequence. Preferably, it refers to the percentage of amino acid residues in the shorter sequence which are identical to amino acid residues in the longer sequence. The degree of sequence identity can be determined according to methods well known in the art using preferably suitable computer algorithms such as CLUSTAL.

When using the Clustal analysis method to determine whether a particular sequence is, for instance, at least 60% identical to a reference sequence default settings may be used or the settings are preferably as follows: Matrix: blosum 30; Open gap penalty: 10.0; Extend gap penalty: 0.05; Delay divergent: 40; Gap separation distance: 8 for comparisons of amino acid sequences. For nucleotide sequence comparisons, the Extend gap penalty is preferably set to 5.0.

In a preferred embodiment ClustalW2 is used for the comparison of amino acid sequences. In the case of pairwise comparisons/alignments, the following settings are preferably chosen: Protein weight matrix: BLOSUM 62; gap open: 10; gap extension: 0.1. In the case of multiple comparisons/alignments, the following settings are preferably chosen: Protein weight matrix: BLOSUM 62; gap open: 10; gap extension: 0.2; gap distance: 5; no end gap. Preferably, the degree of identity is calculated over the complete length of the sequence.

Amino acid residues located at a position corresponding to a position as indicated herein in the amino acid sequence shown in SEQ ID NO:1 can be identified by the skilled person by methods known in the art. For example, such amino acid residues can be identified by aligning the sequence in question with the sequence shown in SEQ ID NO:1 and by identifying the positions which correspond to the above or below indicated positions of SEQ ID NO:1. The alignment can be done with means and methods known to the skilled person, e.g. by using a known computer algorithm such as the Lipman-Pearson method (Science 227 (1985), 1435) or the CLUSTAL algorithm. It is preferred that in such an alignment maximum homology is assigned to conserved amino acid residues present in the amino acid sequences.

In a preferred embodiment ClustalW2 is used for the comparison of amino acid sequences. In the case of pairwise comparisons/alignments, the following settings are preferably chosen: Protein weight matrix: BLOSUM 62; gap open: 10; gap extension: 0.1. In the case of multiple comparisons/alignments, the following settings are preferably chosen: Protein weight matrix: BLOSUM 62; gap open: 10; gap extension: 0.2; gap distance: 5; no end gap.

When the amino acid sequences of MDCs are aligned by means of such a method, regardless of insertions or deletions that occur in the amino acid sequences, the positions of the corresponding amino acid residues can be determined in each of the MDCs.

In the context of the present invention, “substituted with another amino acid residue” means that the respective amino acid residues at the indicated position can be substituted with any other possible amino acid residues, e.g. naturally occurring amino acids or non-naturally occurring amino acids (Brustad and Arnold, *Curr. Opin. Chem. Biol.* 15 (2011), 201-210), preferably with an amino acid residues selected from the group consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. Preferred substitutions for certain positions are indicated further below. Moreover, the term “substituted” or “substitution” also means that the respective amino acid residue at the indicated position is modified.

Such modifications include naturally occurring modifications and non-naturally occurring modifications. Naturally occurring modifications include but are not limited to eukaryotic post-translational modification, such as attachment of functional groups (e.g. acetate, phosphate, hydroxyl, lipids (myristoylation of glycine residues) and carbohydrates (e.g. glycosylation of arginine, asparagine etc.)). Naturally occurring modifications also encompass the change in the chemical structure by citrullination, carbamylation and disulphide bond formation between cysteine residues; attachment of co-factors (FMN or FAD that can be covalently attached) or the attachment of peptides (e.g. ubiquitination or sumoylation).

Non-naturally occurring modifications include, e.g., in vitro modifications such as biotinylation of lysine residue or the inclusion of non-canonical amino acids (see Liu and Schultz, *Annu. Rev. Biochem.* 79 (2010), 413-44 and Wang et al., *Chem. Bio.* 2009 March 27; 16 (3), 323-336; doi:101016/jchembiol.2009.03.001).

In the context of the present invention, “deleted” or “deletion” means that the amino acid at the corresponding position is deleted.

In the context of the present invention, “inserted” or “insertion” means that at the respective position one or two, preferably one amino acid residue is inserted, preferably in front of the indicated position.

In a second aspect, the present invention provides a variant of a 3-methylcrotonic acid decarboxylase (MDC) showing an improved activity in converting 3-methylcrotonic acid into isobutene over the corresponding MDC from which it is

derived, wherein the MDC variant is characterized in that it shows one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 405, 2, 12, 13, 29, 31, 33, 35, 89, 114, 195, 197, 221, 293, 337, 351, 376, 381, 388, 420, 422, 435, 436, 439, 441, 447, 449, 500, 506 and 511 in the amino acid sequence shown in SEQ ID NO:1.

The present invention relates in a preferred embodiment to an MDC variant having an amino acid sequence as shown in SEQ ID NO:1 or an amino acid sequence having at least 60% sequence identity to SEQ ID NO:1, in which one or more amino acid residues at a position selected from the group consisting of positions 405, 2, 12, 13, 29, 31, 33, 35, 89, 114, 195, 197, 221, 293, 337, 351, 376, 381, 388, 420, 422, 435, 436, 439, 441, 447, 449, 500, 506 and 511 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to any of these positions, are substituted with another amino acid residue or deleted or wherein an insertion has been effected at one or more of these positions and wherein said MDC variant has an improved activity in converting 3-methylcrotonic acid into isobutene.

According to one embodiment, the present invention relates to any of the above-described MDC variants having an amino acid sequence as shown in SEQ ID NO:1 or an amino acid sequence having at least 60% sequence identity to SEQ ID NO:1 in which

- (1) an amino acid residue at position 2 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, aspartic acid, phenylalanine, lysine, leucine, asparagine, glutamine or valine; and/or
- (2) an amino acid residue at position 12 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine, alanine or asparagine; and/or
- (3) an amino acid residue at position 13 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, isoleucine, asparagine, serine, valine or tyrosine; and/or
- (4) an amino acid residue at position 29 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine, histidine or serine; and/or

- (5) an amino acid residue at position 31 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid or glycine; and/or
- (6) an amino acid residue at position 33 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (7) an amino acid residue at position 35 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine, serine or threonine; and/or
- (8) an amino acid residue at position 89 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine or phenylalanine; and/or
- (9) an amino acid residue at position 114 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (10) an amino acid residue at position 195 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine, cysteine, phenylalanine, isoleucine, valine, tryptophan or tyrosine; and/or
- (11) an amino acid residue at position 197 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine or methionine; and/or
- (12) an amino acid residue at position 221 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (13) an amino acid residue at position 293 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (14) an amino acid residue at position 337 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine or leucine; and/or
- (15) an amino acid residue at position 351 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine, asparagine, alanine, valine or glycine; and/or
- (16) an amino acid residue at position 376 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or

- (17) an amino acid residue at position 381 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (18) an amino acid residue at position 388 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid; and/or
- (19) an amino acid residue at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, leucine, methionine, proline or glutamine; and/or
- (20) an amino acid residue at position 420 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (21) an amino acid residue at position 422 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine; and/or
- (22) an amino acid residue at position 435 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine; and/or
- (23) an amino acid residue at position 436 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (24) an amino acid residue at position 439 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (25) an amino acid residue at position 441 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine; and/or
- (26) an amino acid residue at position 447 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tryptophan, methionine or tyrosine; and/or
- (27) an amino acid residue at position 449 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, methionine or valine; and/or
- (28) an amino acid residue at position 500 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or

- (29) an amino acid residue at position 506 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or tyrosine; and/or
- (30) an amino acid residue at position 511 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine or isoleucine.

The invention also relates to variants as defined in (1) to (30) hereinabove, wherein the amino acid residue indicated as substituting the amino acid residue at the position in SEQ ID NO: 1 is not that particular amino acid residue but an amino acid residue which is conservative in relation to the indicated substituting amino acid.

Whether an amino acid is conservative with respect to another amino acid can be judged according to means and methods known in the art and as described herein above. One possibility is the PAM 250 matrix; alternatively, the Blosum Family Matrices can be used.

The present invention also relates to an MDC variant as described herein above which has an amino acid sequence as shown in SEQ ID NO:1 or an amino acid sequence having at least 60% sequence identity to SEQ ID NO:1, in which one or more amino acid residues at a position selected from the group consisting of positions 405, 2, 12, 13, 29, 31, 33, 35, 89, 114, 195, 197, 221, 293, 337, 351, 376, 381, 388, 420, 422, 435, 436, 439, 441, 447, 449, 500, 506 and 511 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to any of these positions, are substituted with another amino acid residue or deleted or wherein an insertion has been effected at one or more of these positions and which furthermore shows at least one modification at a position selected from the group consisting of 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15, 25, 30, 34, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 206, 211, 213, 214, 215, 216, 222, 228, 232, 244, 247, 264, 278, 284, 285, 303, 305, 306, 326, 338, 341, 342, 345, 349, 352, 375, 377, 384, 386, 392, 395, 399, 402, 404, 406, 414, 429, 440, 442, 443, 445, 448, 454, 460, 461, 462, 484, 488, 493, 494, 496, 501, 502, 509 and 512 in the amino acid sequence shown in SEQ ID NO:1.

According to one embodiment, such an MDC variant as described herein above which furthermore shows at least one modification at a position selected from the group consisting of 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15, 25, 30, 34, 40, 43, 57, 60, 65,

67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 206, 211, 213, 214, 215, 216, 222, 228, 232, 244, 247, 264, 278, 284, 285, 303, 305, 306, 326, 338, 341, 342, 345, 349, 352, 375, 377, 384, 386, 392, 395, 399, 402, 404, 406, 414, 429, 440, 442, 443, 445, 448, 454, 460, 461, 462, 484, 488, 493, 494, 496, 501, 502, 509 and 512 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to any of these positions is an MDC variant, wherein

- (1) an amino acid residue at position 3 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, glutamic acid, glycine, lysine, proline, tryptophan, cysteine, aspartic acid or tyrosine; and/or
- (2) an amino acid residue at position 4 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid, leucine, methionine, alanine, serine or asparagine; and/or
- (3) an amino acid residue at position 5 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (4) an amino acid residue at position 6 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline; and/or
- (5) an amino acid residue at position 7 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (6) an amino acid residue at position 8 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (7) an amino acid residue at position 9 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, proline or tyrosine; and/or
- (8) an amino acid residue at position 10 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, phenylalanine, lysine, proline, threonine or leucine; and/or
- (9) an amino acid residue at position 11 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine, tyrosine or proline; and/or

- (10) an amino acid residue at position 14 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartic acid; and/or
- (11) an amino acid residue at position 15 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (12) an amino acid residue at position 25 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine, serine or tryptophan; and/or
- (13) an amino acid residue at position 30 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine, histidine or arginine; and/or
- (14) an amino acid residue at position 34 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or isoleucine; and/or
- (15) an amino acid residue at position 40 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or methionine; and/or
- (16) an amino acid residue at position 43 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (17) an amino acid residue at position 57 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (18) an amino acid residue at position 60 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or
- (19) an amino acid residue at position 65 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine or tryptophan; and/or
- (20) an amino acid residue at position 67 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine or valine; and/or
- (21) an amino acid residue at position 69 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or

- (22) an amino acid residue at position 70 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or leucine; and/or
- (23) an amino acid residue at position 71 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or
- (24) an amino acid residue at position 72 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (25) an amino acid residue at position 80 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (26) an amino acid residue at position 84 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (27) an amino acid residue at position 85 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or threonine; and/or
- (28) an amino acid residue at position 86 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine or isoleucine; and/or
- (29) an amino acid residue at position 87 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, cysteine, phenylalanine, isoleucine, leucine, methionine, valine or tryptophan; and/or
- (30) an amino acid residue at position 90 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (31) an amino acid residue at position 91 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (32) an amino acid residue at position 99 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine or proline; and/or
- (33) an amino acid residue at position 101 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or leucine; and/or

- (34) an amino acid residue at position 102 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (35) an amino acid residue at position 103 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, leucine or methionine; and/or
- (36) an amino acid residue at position 105 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, leucine or tryptophan; and/or
- (37) an amino acid residue at position 106 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (38) an amino acid residue at position 108 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with lysine, arginine or tryptophan; and/or
- (39) an amino acid residue at position 111 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (40) an amino acid residue at position 117 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or
- (41) an amino acid residue at position 120 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine or lysine; and/or
- (42) an amino acid residue at position 119 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (43) an amino acid residue at position 126 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline; and/or
- (44) an amino acid residue at position 132 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (45) an amino acid residue at position 141 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartic acid; and/or

- (46) an amino acid residue at position 146 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (47) an amino acid residue at position 149 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine or serine; and/or
- (48) an amino acid residue at position 154 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with lysine; and/or
- (49) an amino acid residue at position 159 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (50) an amino acid residue at position 160 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (51) an amino acid residue at position 162 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline, histidine or asparagine; and/or
- (52) an amino acid residue at position 175 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine, lysine, proline, glutamine, serine, threonine or tryptophan; and/or
- (53) an amino acid residue at position 176 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (54) an amino acid residue at position 187 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (55) an amino acid residue at position 189 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (56) an amino acid residue at position 193 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, threonine or valine; and/or
- (57) an amino acid residue at position 206 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or

- (58) an amino acid residue at position 211 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid; and/or
- (59) an amino acid residue at position 213 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline or leucine; and/or
- (60) an amino acid residue at position 214 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, threonine or valine, histidine, glutamic acid, arginine or phenylalanine; and/or
- (61) an amino acid residue at position 215 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (62) an amino acid residue at position 216 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (63) an amino acid residue at position 222 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (64) an amino acid residue at position 228 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine, alanine, proline, threonine or valine; and/or
- (65) an amino acid residue at position 232 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or
- (66) an amino acid residue at position 244 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (67) an amino acid residue at position 247 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or
- (68) an amino acid residue at position 264 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartic acid; and/or
- (69) an amino acid residue at position 278 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or

- (70) an amino acid residue at position 284 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine or leucine; and/or
- (71) an amino acid residue at position 285 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (72) an amino acid residue at position 303 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine or proline; and/or
- (73) an amino acid residue at position 305 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or aspartic acid; and/or
- (74) an amino acid residue at position 306 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, arginine or serine; and/or
- (75) an amino acid residue at position 326 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or proline; and/or
- (76) an amino acid residue at position 338 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline, alanine or serine; and/or
- (77) an amino acid residue at position 341 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (78) an amino acid residue at position 342 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or
- (79) an amino acid residue at position 345 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (80) an amino acid residue at position 349 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (81) an amino acid residue at position 352 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine or leucine; and/or

- (82) an amino acid residue at position 375 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (83) an amino acid residue at position 377 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine; and/or
- (84) an amino acid residue at position 384 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine; and/or
- (85) an amino acid residue at position 386 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (86) an amino acid residue at position 392 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine or alanine; and/or
- (87) an amino acid residue at position 395 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (88) an amino acid residue at position 399 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine or asparagine; and/or
- (89) an amino acid residue at position 402 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine or histidine; and/or
- (90) an amino acid residue at position 404 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine or tryptophan; and/or
- (91) an amino acid residue at position 406 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamine; and/or
- (92) an amino acid residue at position 414 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (93) an amino acid residue at position 440 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or

- (94) an amino acid residue at position 443 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (95) an amino acid residue at position 448 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine, phenylalanine or tryptophan; and/or
- (96) an amino acid residue at position 454 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or
- (97) an amino acid residue at position 460 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine or proline; and/or
- (98) an amino acid residue at position 461 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine, asparagine or methionine; and/or
- (99) an amino acid residue at position 462 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (100) an amino acid residue at position 484 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or glycine; and/or
- (101) an amino acid residue at position 488 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or asparagine; and/or
- (102) an amino acid residue at position 493 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (103) an amino acid residue at position 494 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (104) an amino acid residue at position 496 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or phenylalanine; and/or
- (105) an amino acid residue at position 429 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or serine; and/or

- (106) an amino acid residue at position 442 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (107) an amino acid residue at position 445 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid or proline; and/or
- (108) an amino acid residue at position 501 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid, methionine, glycine or lysine; and/or
- (109) an amino acid residue at position 502 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (110) an amino acid residue at position 509 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (111) an amino acid residue at position 512 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid, histidine or serine.

The present invention also relates to an MDC variant having an amino acid sequence as shown in SEQ ID NO:1 or an amino acid sequence having at least 60% sequence identity to SEQ ID NO:1, in which one or more amino acid residues at a position selected from the group consisting of positions 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15, 30, 34, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 117, 119, 120, 126, 132, 146, 154, 159, 160, 162, 175, 176, 187, 189, 193, 206, 211, 213, 214, 215, 216, 222, 228, 232, 244, 247, 264, 278, 284, 285, 303, 305, 306, 326, 338, 341, 342, 345, 349, 352, 375, 377, 384, 386, 392, 395, 399, 402, 404, 406, 414, 429, 440, 442, 443, 445, 448, 454, 460, 461, 462, 484, 488, 493, 494, 496, 501, 502, 509 and 512 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to any of these positions, are substituted with another amino acid residue or deleted, wherein said MDC variant has an improved activity in converting 3-methylcrotonic acid into isobutene and wherein

- (1) an amino acid residue at position 3 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, glutamic acid, glycine, lysine, proline, tryptophan, cysteine, aspartic acid or tyrosine; and/or

- (2) an amino acid residue at position 4 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid, leucine, methionine, alanine, serine or asparagine; and/or
- (3) an amino acid residue at position 5 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (4) an amino acid residue at position 6 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline; and/or
- (5) an amino acid residue at position 7 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (6) an amino acid residue at position 8 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (7) an amino acid residue at position 9 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, proline or tyrosine; and/or
- (8) an amino acid residue at position 10 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, phenylalanine, lysine, proline, threonine or leucine; and/or
- (9) an amino acid residue at position 11 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine, tyrosine or proline; and/or
- (10) an amino acid residue at position 14 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartic acid; and/or
- (11) an amino acid residue at position 15 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (12) an amino acid residue at position 30 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine, histidine or arginine; and/or
- (13) an amino acid residue at position 34 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or isoleucine; and/or

- (14) an amino acid residue at position 40 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or methionine; and/or
- (15) an amino acid residue at position 43 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (16) an amino acid residue at position 57 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (17) an amino acid residue at position 60 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or
- (18) an amino acid residue at position 65 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine or tryptophan; and/or
- (19) an amino acid residue at position 67 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine or valine; and/or
- (20) an amino acid residue at position 69 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (21) an amino acid residue at position 70 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or leucine; and/or
- (22) an amino acid residue at position 71 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or
- (23) an amino acid residue at position 72 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (24) an amino acid residue at position 80 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (25) an amino acid residue at position 84 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or

- (26) an amino acid residue at position 85 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or threonine; and/or
- (27) an amino acid residue at position 86 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine or isoleucine; and/or
- (28) an amino acid residue at position 87 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, cysteine, phenylalanine, isoleucine, leucine, methionine, valine or tryptophan; and/or
- (29) an amino acid residue at position 90 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (30) an amino acid residue at position 91 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (31) an amino acid residue at position 99 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine or proline; and/or
- (31) an amino acid residue at position 101 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or leucine; and/or
- (33) an amino acid residue at position 102 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (34) an amino acid residue at position 103 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, leucine or methionine; and/or
- (35) an amino acid residue at position 105 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, leucine or tryptophan; and/or
- (36) an amino acid residue at position 106 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (37) an amino acid residue at position 108 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with lysine, arginine or tryptophan; and/or

- (38) an amino acid residue at position 111 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (39) an amino acid residue at position 117 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or
- (40) an amino acid residue at position 120 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine or lysine; and/or
- (41) an amino acid residue at position 119 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (42) an amino acid residue at position 126 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline; and/or
- (43) an amino acid residue at position 132 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (44) an amino acid residue at position 146 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (45) an amino acid residue at position 154 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with lysine; and/or
- (46) an amino acid residue at position 159 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (47) an amino acid residue at position 160 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (48) an amino acid residue at position 162 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline, histidine or asparagine; and/or
- (49) an amino acid residue at position 175 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine, lysine, proline, glutamine, serine, threonine or tryptophan; and/or

- (50) an amino acid residue at position 176 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (51) an amino acid residue at position 187 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (52) an amino acid residue at position 189 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (53) an amino acid residue at position 193 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, threonine or valine; and/or
- (54) an amino acid residue at position 206 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (55) an amino acid residue at position 211 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid; and/or
- (56) an amino acid residue at position 213 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline or leucine; and/or
- (57) an amino acid residue at position 214 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, threonine or valine, histidine, glutamic acid, arginine or phenylalanine; and/or
- (58) an amino acid residue at position 215 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (59) an amino acid residue at position 216 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (60) an amino acid residue at position 222 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (61) an amino acid residue at position 228 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine, alanine, proline, threonine or valine; and/or

- (62) an amino acid residue at position 232 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or
- (63) an amino acid residue at position 244 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (64) an amino acid residue at position 247 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or
- (65) an amino acid residue at position 264 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartic acid; and/or
- (66) an amino acid residue at position 278 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (67) an amino acid residue at position 284 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine or leucine; and/or
- (68) an amino acid residue at position 285 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (69) an amino acid residue at position 303 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine or proline; and/or
- (70) an amino acid residue at position 305 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or aspartic acid; and/or
- (71) an amino acid residue at position 306 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, arginine or serine; and/or
- (72) an amino acid residue at position 326 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or proline; and/or
- (73) an amino acid residue at position 338 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline, alanine or serine; and/or

- (74) an amino acid residue at position 341 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (75) an amino acid residue at position 342 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or
- (76) an amino acid residue at position 345 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (77) an amino acid residue at position 349 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (78) an amino acid residue at position 352 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine or leucine; and/or
- (79) an amino acid residue at position 375 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (80) an amino acid residue at position 377 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine; and/or
- (81) an amino acid residue at position 384 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine; and/or
- (82) an amino acid residue at position 386 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (83) an amino acid residue at position 392 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine or alanine; and/or
- (84) an amino acid residue at position 395 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (85) an amino acid residue at position 399 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine or asparagine; and/or

- (86) an amino acid residue at position 402 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine or histidine; and/or
- (87) an amino acid residue at position 404 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine or tryptophan; and/or
- (88) an amino acid residue at position 406 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamine; and/or
- (89) an amino acid residue at position 414 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (90) an amino acid residue at position 440 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or
- (91) an amino acid residue at position 443 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (92) an amino acid residue at position 448 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine, phenylalanine or tryptophan; and/or
- (93) an amino acid residue at position 454 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or
- (94) an amino acid residue at position 460 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine or proline; and/or
- (95) an amino acid residue at position 461 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine, asparagine or methionine; and/or
- (96) an amino acid residue at position 462 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (97) an amino acid residue at position 484 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or glycine; and/or

- (98) an amino acid residue at position 488 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or asparagine; and/or
- (99) an amino acid residue at position 493 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (100) an amino acid residue at position 494 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (101) an amino acid residue at position 496 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or phenylalanine; and/or
- (102) an amino acid residue at position 429 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or serine; and/or
- (103) an amino acid residue at position 442 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (104) an amino acid residue at position 445 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid or proline; and/or
- (105) an amino acid residue at position 501 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid, methionine, glycine or lysine; and/or
- (106) an amino acid residue at position 502 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (107) an amino acid residue at position 509 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (108) an amino acid residue at position 512 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid, histidine or serine.

The present invention also relates to an MDC variant having an amino acid sequence as shown in SEQ ID NO:1 or an amino acid sequence having at least 83%, at least

85%, or at least 87%, preferably at least 90% or at least 95%, even more preferred at least 98% sequence identity to SEQ ID NO:1, in which one or more amino acid residues at a position selected from the group consisting of positions 2, 3, 4, 7, 8, 10, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 111, 114, 117, 119, 120, 126, 141, 146, 154, 159, 160, 162, 176, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 381, 384, 386, 388, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 447, 449, 460, 462, 488, 493, 494, 496, 500, 501, 502, 506, 509, and 511 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to any of these positions, are substituted with another amino acid residue or deleted or wherein an insertion has been effected at one or more of these positions and wherein said MDC variant has an improved activity in converting 3-methylcrotonic acid into isobutene.

According to one embodiment, the present invention relates to any of these MDC variants having an amino acid sequence as shown in SEQ ID NO:1 or an amino acid sequence having at least 83%, at least 85%, or at least 87%, preferably at least 90% or at least 95%, even more preferred at least 98% sequence identity to SEQ ID NO:1 in which

- (1) an amino acid residue at position 2 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, aspartic acid, phenylalanine, lysine, leucine, asparagine, glutamine, cysteine or valine; and/or
- (2) an amino acid residue at position 3 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, glutamic acid, glycine, lysine, proline, tryptophan, aspartic acid or tyrosine; and/or
- (3) an amino acid residue at position 4 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid, leucine, methionine, alanine, serine or asparagine; and/or
- (4) an amino acid residue at position 7 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (5) an amino acid residue at position 8 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or

- (6) an amino acid residue at position 10 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, phenylalanine, lysine, proline, threonine or leucine; and/or
- (7) an amino acid residue at position 12 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine or asparagine or alanine; and/or
- (8) an amino acid residue at position 13 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, isoleucine, asparagine, serine, valine or tyrosine; and/or
- (9) an amino acid residue at position 14 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartic acid; and/or
- (10) an amino acid residue at position 15 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (11) an amino acid residue at position 25 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine, serine or tryptophan; and/or
- (12) an amino acid residue at position 29 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine, histidine or serine; and/or
- (13) an amino acid residue at position 30 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine, histidine or arginine; and/or
- (14) an amino acid residue at position 31 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid or glycine or lysine; and/or
- (15) an amino acid residue at position 33 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (16) an amino acid residue at position 34 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or isoleucine; and/or
- (17) an amino acid residue at position 35 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine, serine or threonine; and/or

- (18) an amino acid residue at position 40 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or methionine; and/or
- (19) an amino acid residue at position 43 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (20) an amino acid residue at position 57 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (21) an amino acid residue at position 60 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or
- (22) an amino acid residue at position 65 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine or tryptophan; and/or
- (23) an amino acid residue at position 67 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine or valine; and/or
- (24) an amino acid residue at position 69 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (25) an amino acid residue at position 70 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or leucine; and/or
- (26) an amino acid residue at position 71 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or
- (27) an amino acid residue at position 72 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (28) an amino acid residue at position 80 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (29) an amino acid residue at position 84 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or

- (30) an amino acid residue at position 85 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or threonine; and/or
- (31) an amino acid residue at position 86 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine or isoleucine; and/or
- (32) an amino acid residue at position 87 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, cysteine, phenylalanine, isoleucine, leucine, methionine, valine or tryptophan; and/or
- (33) an amino acid residue at position 89 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine or phenylalanine; and/or
- (34) an amino acid residue at position 90 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (35) an amino acid residue at position 91 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (36) an amino acid residue at position 99 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine or proline; and/or
- (37) an amino acid residue at position 101 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or leucine; and/or
- (38) an amino acid residue at position 102 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (39) an amino acid residue at position 103 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, leucine or methionine; and/or
- (40) an amino acid residue at position 105 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, leucine or tryptophan; and/or
- (41) an amino acid residue at position 111 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or

- (42) an amino acid residue at position 117 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or
- (43) an amino acid residue at position 114 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (44) an amino acid residue at position 119 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (45) an amino acid residue at position 120 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (46) an amino acid residue at position 126 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline; and/or
- (47) an amino acid residue at position 141 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartic acid; and/or
- (48) an amino acid residue at position 146 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (49) an amino acid residue at position 159 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (50) an amino acid residue at position 160 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (51) an amino acid residue at position 162 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline, histidine or asparagine; and/or
- (52) an amino acid residue at position 176 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (53) an amino acid residue at position 189 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or

- (54) an amino acid residue at position 193 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, threonine or valine; and/or
- (55) an amino acid residue at position 195 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine, cysteine, phenylalanine, isoleucine, valine, tryptophan or tyrosine; and/or
- (56) an amino acid residue at position 197 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine or methionine; and/or
- (57) an amino acid residue at position 206 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (58) an amino acid residue at position 211 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid; and/or
- (59) an amino acid residue at position 213 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline or leucine; and/or
- (60) an amino acid residue at position 214 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, threonine, histidine, glutamic acid, phenylalanine, arginine or valine; and/or
- (61) an amino acid residue at position 215 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (62) an amino acid residue at position 216 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (63) an amino acid residue at position 221 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (64) an amino acid residue at position 222 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (65) an amino acid residue at position 228 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine, alanine, proline, threonine or valine; and/or

- (66) an amino acid residue at position 232 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or
- (67) an amino acid residue at position 244 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (68) an amino acid residue at position 247 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or
- (69) an amino acid residue at position 264 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartic acid; and/or
- (70) an amino acid residue at position 278 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (71) an amino acid residue at position 284 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine or leucine; and/or
- (72) an amino acid residue at position 285 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (73) an amino acid residue at position 293 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (74) an amino acid residue at position 303 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine or proline; and/or
- (75) an amino acid residue at position 305 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or aspartic acid; and/or
- (76) an amino acid residue at position 306 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, arginine or serine; and/or
- (77) an amino acid residue at position 326 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or proline; and/or

- (78) an amino acid residue at position 337 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine or leucine; and/or
- (79) an amino acid residue at position 338 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline, alanine or serine; and/or
- (80) an amino acid residue at position 341 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (81) an amino acid residue at position 342 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or
- (82) an amino acid residue at position 345 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (83) an amino acid residue at position 349 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (84) an amino acid residue at position 352 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine or leucine; and/or
- (85) an amino acid residue at position 351 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine, asparagine, alanine or valine or glycine; and/or
- (86) an amino acid residue at position 375 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (88) an amino acid residue at position 376 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (89) an amino acid residue at position 381 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (90) an amino acid residue at position 384 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine; and/or

- (91) an amino acid residue at position 386 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (92) an amino acid residue at position 388 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid; and/or
- (93) an amino acid residue at position 395 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (94) an amino acid residue at position 399 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine or asparagine; and/or
- (95) an amino acid residue at position 402 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine or histidine; and/or
- (96) an amino acid residue at position 404 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine or tryptophane; and/or
- (97) an amino acid residue at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, leucine, methionine, proline or glutamine; and/or
- (98) an amino acid residue at position 406 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamine; and/or
- (99) an amino acid residue at position 414 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (100) an amino acid residue at position 420 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (101) an amino acid residue at position 422 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine; and/or
- (102) an amino acid residue at position 429 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or serine; and/or

- (103) an amino acid residue at position 435 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine; and/or
- (104) an amino acid residue at position 436 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (105) an amino acid residue at position 439 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (106) an amino acid residue at position 440 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or
- (107) an amino acid residue at position 441 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine; and/or
- (108) an amino acid residue at position 442 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (109) an amino acid residue at position 443 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (110) an amino acid residue at position 447 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tryptophan, methionine or tyrosine; and/or
- (111) an amino acid residue at position 449 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, methionine or valine; and/or
- (112) an amino acid residue at position 460 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine or proline; and/or
- (113) an amino acid residue at position 462 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (114) an amino acid residue at position 488 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or asparagine; and/or

- (115) an amino acid residue at position 493 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (116) an amino acid residue at position 494 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (117) an amino acid residue at position 496 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or phenylalanine; and/or
- (118) an amino acid residue at position 500 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or
- (119) an amino acid residue at position 501 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid, glycine or methionine or lysine; and/or
- (120) an amino acid residue at position 502 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (121) an amino acid residue at position 506 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or tyrosine; and/or
- (122) an amino acid residue at position 509 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (123) an amino acid residue at position 511 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine or isoleucine.

The invention also relates to variants as defined in (1) to (123) hereinabove, wherein the amino acid residue indicated as substituting the amino acid residue at the position in SEQ ID NO: 1 is not that particular amino acid residue but an amino acid residue which is conservative in relation to the indicated substituting amino acid.

Whether an amino acid is conservative with respect to another amino acid can be judged according to means and methods known in the art and as described herein above. One possibility is the PAM 250 matrix; alternatively, the Blosum Family Matrices can be used.

The present invention also relates to an MDC variant having an amino acid sequence as shown in SEQ ID NO:1 or an amino acid sequence having at least 95%, preferably at least 97%, even more preferred at least 98% sequence identity to SEQ ID NO:1, in which one or more amino acid residues at a position selected from the group consisting of positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to any of these positions, are substituted with another amino acid residue or deleted or wherein an insertion has been effected at one or more of these positions and wherein said MDC variant has an improved activity in converting 3-methylcrotonic acid into isobutene.

According to one embodiment, the present invention relates to any of these MDC variants having an amino acid sequence as shown in SEQ ID NO:1 or an amino acid sequence having at least 95%, preferably at least 97%, even more preferred at least 98% sequence identity to SEQ ID NO:1 in which

- (1) an amino acid residue at position 2 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, aspartic acid, phenylalanine, lysine, leucine, asparagine, glutamine, cysteine or valine; and/or
- (2) an amino acid residue at position 3 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, glutamic acid, glycine, lysine, proline, tryptophan, aspartic acid or tyrosine; and/or
- (3) an amino acid residue at position 4 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid, leucine, methionine, alanine, serine or asparagine; and/or
- (4) an amino acid residue at position 5 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or

- (5) an amino acid residue at position 6 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline; and/or
- (6) an amino acid residue at position 7 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (7) an amino acid residue at position 8 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (8) an amino acid residue at position 9 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, proline or tyrosine; and/or
- (9) an amino acid residue at position 10 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, phenylalanine, lysine, proline, threonine or leucine; and/or
- (10) an amino acid residue at position 11 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine, tyrosine or proline; and/or
- (11) an amino acid residue at position 12 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine or asparagine or alanine; and/or
- (12) an amino acid residue at position 13 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, isoleucine, asparagine, serine, valine or tyrosine; and/or
- (13) an amino acid residue at position 14 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartic acid; and/or
- (14) an amino acid residue at position 15 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (15) an amino acid residue at position 25 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine, serine or tryptophan; and/or
- (16) an amino acid residue at position 29 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine, histidine or serine; and/or

- (17) an amino acid residue at position 30 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine, histidine or arginine; and/or
- (18) an amino acid residue at position 31 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid or glycine or lysine; and/or
- (19) an amino acid residue at position 33 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (20) an amino acid residue at position 34 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or isoleucine; and/or
- (21) an amino acid residue at position 35 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine, serine or threonine; and/or
- (22) an amino acid residue at position 40 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or methionine; and/or
- (23) an amino acid residue at position 43 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (24) an amino acid residue at position 57 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (25) an amino acid residue at position 60 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or
- (26) an amino acid residue at position 65 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine or tryptophan; and/or
- (27) an amino acid residue at position 67 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine or valine; and/or
- (28) an amino acid residue at position 69 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or

- (29) an amino acid residue at position 70 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or leucine; and/or
- (30) an amino acid residue at position 71 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or
- (31) an amino acid residue at position 72 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (32) an amino acid residue at position 80 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (33) an amino acid residue at position 84 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (34) an amino acid residue at position 85 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or threonine; and/or
- (35) an amino acid residue at position 86 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine or isoleucine; and/or
- (36) an amino acid residue at position 87 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, cysteine, phenylalanine, isoleucine, leucine, methionine, valine or tryptophan; and/or
- (37) an amino acid residue at position 89 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine or phenylalanine; and/or
- (38) an amino acid residue at position 90 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (39) an amino acid residue at position 91 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (40) an amino acid residue at position 99 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine or proline; and/or

- (41) an amino acid residue at position 101 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or leucine; and/or
- (42) an amino acid residue at position 102 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (43) an amino acid residue at position 103 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, leucine or methionine; and/or
- (44) an amino acid residue at position 105 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, leucine or tryptophan; and/or
- (45) an amino acid residue at position 106 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (46) an amino acid residue at position 108 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with lysine, arginine or tryptophan; and/or
- (47) an amino acid residue at position 111 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (48) an amino acid residue at position 117 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or
- (49) an amino acid residue at position 114 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (50) an amino acid residue at position 119 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (51) an amino acid residue at position 120 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (52) an amino acid residue at position 126 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline; and/or

- (53) an amino acid residue at position 132 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (54) an amino acid residue at position 141 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartic acid; and/or
- (55) an amino acid residue at position 146 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (56) an amino acid residue at position 149 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine or serine; and/or
- (57) an amino acid residue at position 159 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (58) an amino acid residue at position 160 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (59) an amino acid residue at position 162 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline, histidine or asparagine; and/or
- (60) an amino acid residue at position 175 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine, lysine, proline, glutamine, serine, threonine, or tryptophan; and/or
- (61) an amino acid residue at position 176 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (62) an amino acid residue at position 187 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (63) an amino acid residue at position 189 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (64) an amino acid residue at position 193 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, threonine or valine; and/or

- (65) an amino acid residue at position 195 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine, cysteine, phenylalanine, isoleucine, valine, tryptophan or tyrosine; and/or
- (66) an amino acid residue at position 197 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine or methionine; and/or
- (67) an amino acid residue at position 206 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (68) an amino acid residue at position 211 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid; and/or
- (69) an amino acid residue at position 213 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline or leucine; and/or
- (70) an amino acid residue at position 214 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, threonine, histidine, glutamic acid, phenylalanine, arginine or valine; and/or
- (71) an amino acid residue at position 215 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (72) an amino acid residue at position 216 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (73) an amino acid residue at position 221 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (74) an amino acid residue at position 222 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (75) an amino acid residue at position 228 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine, alanine, proline, threonine or valine; and/or
- (76) an amino acid residue at position 232 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or

- (77) an amino acid residue at position 244 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (78) an amino acid residue at position 247 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or
- (79) an amino acid residue at position 264 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartic acid; and/or
- (80) an amino acid residue at position 278 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (81) an amino acid residue at position 284 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine or leucine; and/or
- (82) an amino acid residue at position 285 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (83) an amino acid residue at position 293 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (84) an amino acid residue at position 303 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine or proline; and/or
- (85) an amino acid residue at position 305 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or aspartic acid; and/or
- (86) an amino acid residue at position 306 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, arginine or serine; and/or
- (87) an amino acid residue at position 326 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or proline; and/or
- (88) an amino acid residue at position 337 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine or leucine; and/or

- (89) an amino acid residue at position 338 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline, alanine or serine; and/or
- (90) an amino acid residue at position 341 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (91) an amino acid residue at position 342 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or
- (92) an amino acid residue at position 345 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (93) an amino acid residue at position 349 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (94) an amino acid residue at position 352 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine or leucine; and/or
- (95) an amino acid residue at position 351 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine, asparagine, alanine or valine or glycine; and/or
- (96) an amino acid residue at position 375 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (97) an amino acid residue at position 377 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine; and/or
- (98) an amino acid residue at position 376 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (99) an amino acid residue at position 381 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (100) an amino acid residue at position 384 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine; and/or

- (101) an amino acid residue at position 386 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (102) an amino acid residue at position 388 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid; and/or
- (103) an amino acid residue at position 392 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine or alanine; and/or
- (104) an amino acid residue at position 395 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (105) an amino acid residue at position 399 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine or asparagine; and/or
- (106) an amino acid residue at position 402 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine or histidine; and/or
- (107) an amino acid residue at position 404 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine or tryptophane; and/or
- (108) an amino acid residue at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, leucine, methionine, proline or glutamine; and/or
- (109) an amino acid residue at position 406 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamine; and/or
- (110) an amino acid residue at position 414 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (111) an amino acid residue at position 420 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (112) an amino acid residue at position 422 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine; and/or

- (113) an amino acid residue at position 429 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or serine; and/or
- (114) an amino acid residue at position 435 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine; and/or
- (115) an amino acid residue at position 436 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (116) an amino acid residue at position 439 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (117) an amino acid residue at position 440 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or
- (118) an amino acid residue at position 441 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine; and/or
- (119) an amino acid residue at position 442 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (120) an amino acid residue at position 443 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (121) an amino acid residue at position 445 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid or proline; and/or
- (122) an amino acid residue at position 447 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tryptophan, methionine or tyrosine; and/or
- (123) an amino acid residue at position 448 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine, phenylalanine or tryptophan; and/or
- (124) an amino acid residue at position 449 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, methionine or valine; and/or

- (125) an amino acid residue at position 454 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or
- (126) an amino acid residue at position 460 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine or proline; and/or
- (127) an amino acid residue at position 461 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine, asparagine or methionine; and/or
- (128) an amino acid residue at position 462 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (129) an amino acid residue at position 484 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or glycine; and/or
- (130) an amino acid residue at position 488 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or asparagine; and/or
- (131) an amino acid residue at position 493 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (132) an amino acid residue at position 494 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (133) an amino acid residue at position 496 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or phenylalanine; and/or
- (134) an amino acid residue at position 500 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or
- (135) an amino acid residue at position 501 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid, glycine or methionine or lysine; and/or
- (136) an amino acid residue at position 502 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or

- (137) an amino acid residue at position 506 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or tyrosine; and/or
- (138) an amino acid residue at position 509 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (139) an amino acid residue at position 511 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine or isoleucine; and/or
- (140) an amino acid residue at position 512 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine, histidine or serine.

The invention also relates to variants as defined in (1) to 140) hereinabove, wherein the amino acid residue indicated as substituting the amino acid residue at the position in SEQ ID NO: 1 is not that particular amino acid residue but an amino acid residue which is conservative in relation to the indicated substituting amino acid.

Whether an amino acid is conservative with respect to another amino acid can be judged according to means and methods known in the art and as described herein above. One possibility is the PAM 250 matrix; alternatively, the Blosun Family Matrices can be used.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454,

460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: T405M, T405F, T405L, T405Q or T405P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 2 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: S2Q, S2A, S2K, S2L, S2V, S2F, or S2N.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 195 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342,

345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: L195M, L195C, L195I, L195W, L195Y, L195V, or L195F.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 3 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: S3A, S3Y, S3W, S3G, S3P, or S3E.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 449 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154,

159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: L449I, L449M or L449V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 4 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: T4E, T4M, or T4L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 293 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31,

33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: M293L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 40 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: V40M or V40I.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 35 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31,

33, 34, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: D35M, D35T or D35S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 422 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: K422M.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 285 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions

corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: A285L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 448 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: Q448W or Q448S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 34 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein

these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: V34A or V34I.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 12 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: D12S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 500 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein

these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: L500A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 351 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: D351R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 214 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein

these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: Q214T, Q214A or Q214V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 9 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: E9Y, E9H or E9P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 447 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a

variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: F447W or F447Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 114 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: L114S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 13 in the amino acid sequence shown in

SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: P13N, P13I, P13Y, P13S or P13H.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 11 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: F11P, or F11L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the

deletion/insertion/substitution is at position 43 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: D43R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 420 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: D420L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the

deletion/insertion/substitution is at position 439 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: V439L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 337 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: I337M.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the

deletion/insertion/substitution is at position 506 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: L506I.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 120 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: P120S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the

deletion/insertion/substitution is at position 429 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: T429S, or T429A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 436 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: T436N.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized

in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 435 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: G435M.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 149 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: A149V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized

in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 29 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: Q29N, or Q29S. In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 10 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: A10H.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized

in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 31 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: N31G.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 25 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: E25N.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized

in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 501 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: N501K.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 197 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: I197F.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized

in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 146 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: A146S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 442 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: D442T.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized

in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 445 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: V445P or V445E.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 33 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: L33I.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 381 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: A381R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 221 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: L221C.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 141 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and wherein such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: N141D.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least one deletion, substitution and/or insertion wherein the deletion/insertion/substitution is at position 441 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitution in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to this position: F441Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least two deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 3 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 511 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: S3K-L511M.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least two deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 4 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 91 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405,

406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: T4N-F91L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least two deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 3 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 284 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: S3K-M284Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least two deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 2 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 89 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 90, 91, 99, 101,

102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: S2D-E89S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least two deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 31 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 501 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: N31E-N501E.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least two deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 376 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 388 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a

variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 377, 381, 384, 386, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: T376I-A388E.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least two deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 29 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 351 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: Q29N-D351N.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least two deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 29 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 448 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: Q29N-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least two deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 420 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404,

406, 414, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: T405M-D420L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least two deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 351 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: D351N-T405M.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least two deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 29 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions

corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: Q29N-T405M.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least two deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 448 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: T405M-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least three deletions, substitutions and/or insertions wherein one

deletion/insertion/substitution is at position 351 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, another deletion/insertion/substitution is at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 445 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: D351R-T405M-V445P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least three deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 351 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, another deletion/insertion/substitution is at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 448 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341,

342, 345, 349, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: D351R-T405M-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least three deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 25 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, another deletion/insertion/substitution is at position 31 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 29, 30, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-T405M.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least three deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 29 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, another deletion/insertion/substitution is at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another

deletion/insertion/substitution is at position 429 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 406, 414, 420, 422, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: Q29N-T405M-T429A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least three deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 29 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, another deletion/insertion/substitution is at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 448 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: Q29N-T405M-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least three deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 31 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, another deletion/insertion/substitution is at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 445 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: N31G-T405M-V445P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least three deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 25 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, another deletion/insertion/substitution is at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 429 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein

these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 406, 414, 420, 422, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-T405M-T429A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that contains at least three deletions, substitutions and/or insertions wherein one deletion/insertion/substitution is at position 31 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, another deletion/insertion/substitution is at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position and another deletion/insertion/substitution is at position 448 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position. Preferably, such a variant further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: N31G-T405M-Q448W.

In other preferred embodiments, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it has multiple mutations. As it is exemplified in the examples further below, variants have been found bearing multiple mutations which exhibit an increase in the reaction rate of the conversion of 3-methylcrotonic acid into isobutene. These variants bearing multiple mutations are summarized in the following:

Accordingly, in a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 351, 405, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: D351R-T405M-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 29, 351, 405, 429, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: Q29N-D351R-T405M-T429A-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 29, 405, 435, and 445 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-Q29N-T405M-G435M-V445P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 29, 31, 405, 435, and 445 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions:

E25N-Q29N-N31G-T405M-G435M-V445P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 29, 31, 405, and 429 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: Q29H-N31G-T405M-T429A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 29, 31, 351, 405, and 445 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-Q29N-N31G-D351R-T405M-V445P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 29, 351, 405, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: Q29N-D351N-T405M-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 31, 351, 405, 429, and 445 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: N31G-D351R-T405M-T429A-V445P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 29, 31, 405, and 429 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these

positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-Q29N-N31G-T405M-T429A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 29, 31, 351, 405, and 429 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: Q29N-N31G-D351R-T405M-T429A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 31, 405, 420, 429, 445 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: N31G-T405M-D420L-T429A-V445P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 29, 351, 405, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-Q29H-D351R-T405M-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 351, 405, 435, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-D351R-T405M-G435M-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the

deletions/insertions/substitutions are at positions 86, 405, 429, 435, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: S86N-T405M-T429A-G435M-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 29, 31, 351, 405, and 429 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-Q29N-N31G-D351R-T405M-T429A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 29, 351, 405, 414, 429, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: Q29N-D351R-T405M-D414N-T429A-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 31, 351, 405, 429, 435, and 445 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: N31G-D351R-T405M-T429A-G435M-V445P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 405, 429, and 445 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-T405M-T429A-V445P.

In a preferred embodiment, the MDC variant according to the invention showing an

improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 351, 405, 429, and 445 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-D351R-T405M-T429A-V445P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 405, 429, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-T405M-T429A-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 405, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-T405M-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 29, 405, 429, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-Q29N-T405M-T429A-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 29, 31, 351, 405, 429, 435, and 445 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these

positions: Q29N-N31G-D351R-T405M-T429A-G435M-V445P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 31, 405, 420, 429, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: N31G-T405M-D420L-T429A-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 29, 31, 351, 405, 429, and 445 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: Q29N-N31G-D351G-T405M-T429A-V445P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 29, 405, 429, 435, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: Q29N-T405M-T429A-G435M-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 29, 31, 405, 429, and 445 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these

positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: Q29N-N31G-T405M-T429A-V445P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 435, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-G435M-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 31, 351, 405, 429, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: N31G-D351R-T405M-T429A-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 12, 29, 31, 405, 429, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: D12N-Q29N-N31G-T405M-T429A-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 29, 31, 405, 429, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: Q29N-N31G-T405M-T429A-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the

deletions/insertions/substitutions are at positions 25, 405, 429, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-T405M-T429A-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 405, 429, 435, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-T405M-T429A-G435M-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 29, 31, 405, 429, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-Q29N-N31G-T405M-T429A-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 405, 429, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-T405M-T429A-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an

improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 120 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-P120K.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 303 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-H303S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445 and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351G-T405M-T429A-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 264 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-N264D.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448,

and 392 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-R392L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 221 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-L221C.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 305 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-G305A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 44, 448, and 85 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-S85A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 214 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions

corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-Q214V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 402 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-P402V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 228 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-T228L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 119 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-I119T.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 10 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-A10L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 484 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-S484A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 214 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-Q214E.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 214 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-Q214A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized

in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 211 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-A211E.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 501 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-N501M.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 214 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-Q214F.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 228 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-T228V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448,

and 57 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-D57N.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 512 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-D512E.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 484 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-S484G.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 149 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-A149V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 3, 86, 162, 384, and 392 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the

following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-S3C-S86I-Q162P-T384Y-R392A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 162, 228, and 392 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-Q162N-T228L-R392A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 162, and 392 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-Q162N-R392A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 162, 228, 392, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-Q162N-T228L-R392A-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 85, 162, 214, 228, 338, and 461 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-

V445P-Q448W-S85A-Q162N-Q214H-T228L-G338P-I461V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 175, 228, 392, 399, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-C175G-T228L-R392A-T399R-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 85, 162, 175, 228, 338, 399, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-S85A-Q162N-C175G-T228L-G338P-T399R-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 85, 162, 228, 338, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-S85A-Q162N-T228L-G338P-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 85, 162, 175, 228, 338, 399, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-S85A-Q162N-C175G-T228L-G338P-T399R-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an

improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 85, 162, 228, 338, 392, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-S85A-Q162N-T228L-G338P-R392A-I461M-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 162, 228, 338, 392, and 461 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-Q162N-T228L-G338P-R392A-I461V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 162, 175, 228, 338, 392, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-Q162N-C175G-T228L-G338P-R392A-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 85, 175, 228, 338, 392, 399, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-S85A-C175G-T228L-G338P-R392A-T399R-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the

deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 85, 162, 175, 228, 338, 392, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-S85A-Q162N-C175G-T228L-G338P-R392A-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 228, 338, 392, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-T228L-G338P-R392A-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 305, and 402 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-G305A-P402V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 305, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-G305A-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 305, 402, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions

corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-G305A-P402V-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 8 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-S8N.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 10 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-A10H.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 117 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-N117A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 132 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions

corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-V132C.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 175 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-C175G.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 175 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-C175K.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 175 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-C175S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 187 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-S187T.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 193 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-A193T.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 197 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-I197M.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448 and 222 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-A222C.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 228 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-T228A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the

deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 247 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-V247A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 342 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-A342G.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 399 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-T399N.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 440 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-F440V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 460 in the amino acid sequence shown in SEQ ID NO:1 or at positions

corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-A460P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 488 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-K488A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 501 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-N501G.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 502 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-S502N.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, and 506 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions

corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-L506Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 15 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-P15T.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 30 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-D30G.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 30 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-D30H.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 30 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-D30R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 65 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-N65W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 65 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-N65L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 70 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-K70L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 72 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-G72R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized

in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 80 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-P80L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 87 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-P87V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 87 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-P87I.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 90 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-R90L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351,

392, 405, 429, 445, 448, 461, 494, 103 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-T103L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 105 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-S105W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494 and 105 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-S105F.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 108 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-D108R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the

deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 108 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-D108W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 126 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-I126P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175P-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 176 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-W176F.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized

in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 213 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-G213L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 213 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-G213P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 305 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-G305D.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 306 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-P306R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 306 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-P306S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 326P in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-C326P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338S-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 351, 392, 405, 429, 445, 448, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 341 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-A341I.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351V-R392A-T405M-T429A-V445P-Q448W-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 402 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-P402H.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 406 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-

C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-I406Q.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461N-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 103, and 111 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-T103I-D111C.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 14, and 87 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-E14D-P87A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 176, and 511 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID

NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-W176F-L511I.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 352 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25W-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-A352L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 352 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25S-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-A352G.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 278, and 326 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-T278I-C326P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 341, 386, and 395 in the amino acid sequence

shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-A341I-G386N-D395C.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 349 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351A-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-C349S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 7, and 176 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-K7R-W176F.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 84, and 493 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-R84C-K493R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the

deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 87, and 159 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-P87W-K159C.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 87, 488, and 496 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-P87C-K488N-Q496A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 89, and 496 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-E89F-Q496F.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 377, 402, 404, 405, 429, 445, and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-K377H-P402V-F404Y-T405M-T429A-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized

in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 305, 377, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-G305A-K377H-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 305, 306, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-G305A-P306F-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 377, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-K377H-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 306, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-P306F-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 306, and 377 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-P306F-K377H.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 338, 351, 392, 405, 429, 445, 448, 461, 494, 306, 377, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G338P-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-P306F-K377H-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 12 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at

positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-D12A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 35 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-D35T.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 60 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-A60V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 70 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-K70I.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 70 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-K70L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 87 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-P87F.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448 and 87 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-P87L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 87 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-P87M.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 87 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-P87V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized

in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 87 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-P87W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 99 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-A99P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 101 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-P101I.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 101 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-P101L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405,

429, 445, 448, and 102 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-P102L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 103 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-T103L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 105 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-S105L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 108 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-D108R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 189 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the

following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-K189I.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 193 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-A193I.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 215 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-E215C.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 244 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-A244F.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 326 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at

positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-C326A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 375 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-D375L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 443 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-D443N.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 460 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-A460F.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, 102, and 484 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-P102L-S484A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, 108, and 160 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-D108K-Y160F.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, 11, and 512 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-F11Y-D512S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 351, 405, 429, 445, 448, 85, 103, 162, 175, 228, 338, 392, and 461 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-D351R-T405M-T429A-V445P-Q448W-S85T-T103M-Q162H-C175W-T228P-G338A-R392A-I461M.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 445, 448, and 87 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-V445P-Q448W-P87F.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized

in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions E25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 3 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-S3G.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 4 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-T4A.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 5 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-T5S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 10 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-A10F.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405,

429, 445, 448, and 10 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-A10P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 10 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-A10T.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 67 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-I67R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 67 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-I67V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 71 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the

following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-D71G.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 99 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-A99N.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 149 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-A149S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 154 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-Q154K.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 193 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at

positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-A193T.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 193 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-A193V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 206 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-Q206F.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 214 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-Q214R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 232 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-A232V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 284 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-M284L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 40, 404, 405, 429, 445, 448, and 303 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-H303P.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 402, 404, 405, 429, 445, 448, and 462N in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-P402V-F404Y-T405M-T429A-V445P-Q448W-K462N.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 351, 392, 405, 429, 445, 448, 494, 305, 402, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-D351R-R392A-T405M-T429A-V445P-Q448W-S494R-G305A-P402V-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 351, 392, 405, 429, 445, 448, 461, 494, 305, 402, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-G305A-P402V-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 351, 392, 405, 429, 445, 448, 494, 228, 305, 402, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-D351R-R392A-T405M-T429A-V445P-Q448W-S494R-L228T-G305A-P402V-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 351, 392, 405, 429, 445, 448, 494, 305, 377, 402, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-D351R-R392A-T405M-T429A-V445P-Q448W-S494R-G305A-K377H-P402V-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 351, 392, 405, 429, 445, 448, 461, 494, 228, 305, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-

C175G-T228L-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-L228T-G305A-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 351, 392, 405, 429, 445, 448, 461, 494, 305, 377, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-G305A-K377H-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 351, 392, 405, 429, 445, 448, 461, 494, 305, and 404 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-G305A-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 351, 392, 405, 429, 445, 448, 461, 494, 305, 377, 402, and 404Y in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-G305A-K377H-P402V-F404Y.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 351, 392, 405, 429, 445, 448, 461, 494, 305, and 377 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a

variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-D351R-R392A-T405M-T429A-V445P-Q448W-I461V-S494R-G305A-K377H.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, 494, and 2 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R-S2N.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 46, 494, and 10 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R-A10K.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, 494, and 13 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R-P13S.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351,

377, 392, 402, 404, 405, 429, 445, 448, 461, 494, and 69 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R-A69N.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, 494, and 106 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R-A106T.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175T-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175K-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized

in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175Q-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, 494, and 216 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R-E216N.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, 494, and 345 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R-I345L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, 494, and 454 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R-S454G.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, 494, 3, and 509 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R-S3D-K509L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, 494, 4, and 13 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R-T4S-P13V.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, 494, 6, and 512 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R-Y6P-D512H.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-

Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405F-T429A-V445P-Q448W-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, 494, and 454 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405F-T429A-V445P-Q448W-I461V-S494R-S454G.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, 494, and 447 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404W-T405M-T429A-V445P-Q448W-I461V-S494R-F447M.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, 494, and 337 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448W-I461V-S494R-I337L.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, 494, and 197 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions.

Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448F-I461V-S494R-I197M.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 85, 162, 175, 228, 305, 351, 377, 392, 402, 404, 405, 429, 445, 448, 461, and 494 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-S85A-Q162N-C175G-T228L-G305A-D351R-K377H-R392A-P402V-F404Y-T405M-T429A-V445P-Q448F-I461V-S494R.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 377, 402, 404, 405, 429, 445, 448 and 454 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-K377H-P402V-F404Y-T405M-T429A-V445P-Q448W-S454G.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 377, 402, 404, 405, 429, 445 and 448 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-K377H-P402V-F404Y-T405F-T429A-V445P-Q448W.

In a preferred embodiment, the MDC variant according to the invention showing an improved activity in converting 3-methylcrotonic acid into isobutene is characterized in that it comprises deletions, substitutions and/or insertions wherein the deletions/insertions/substitutions are at positions 25, 31, 305, 351, 377, 402, 404, 405, 429, 445, 448 and 454 in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions. Preferably, such a variant has the

following substitutions in the amino acid sequence shown in SEQ ID NO:1 or at positions corresponding to these positions: E25N-N31G-G305A-D351R-K377H-P402V-F404Y-T405F-T429A-V445P-Q448W-S454G.

Preferably, any of the above described variants having multiple mutations further has one or more substitutions, deletions and/or insertions in comparison to the corresponding sequence from which it is derived and wherein these substitutions, deletions and/or insertions occur at one or more of the positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1.

In a third aspect, the present invention provides a variant of a 3-methylcrotonic acid decarboxylase (MDC) showing an improved activity in converting 3-methylcrotonic acid into isobutene over the corresponding MDC from which it is derived, wherein the MDC variant is characterized in that it comprises a substitution, deletion and/or insertion in comparison to the corresponding sequence from which it is derived and wherein this substitution, deletion and/or insertion occurs at position 5 in the following amino acid motif of an MDC:

K-X₂-G-X₄-X₅-X₆-H-R-X₉-X₁₀-X₁₁-X₁₂-G,

wherein the amino acid X₂ at position 2 is an amino acid selected from the group consisting of A, P and V,

wherein the amino acid x₄ at position 4 is any amino acid,

wherein the amino acid X₅ at position 5 is an amino acid selected from the group consisting of A, P and T,

wherein the amino acid X₆ at position 6 is an amino acid selected from the group consisting of F, I and M,

wherein the amino acid X₉ at position 9 is an amino acid selected from the group consisting of I and L,

wherein the amino acid X₁₀ at position 10 is an amino acid selected from the group consisting of A, I, L and V,

wherein the amino acid X_{11} at position 11 is an amino acid selected from the group consisting of I, L and V,

wherein the amino acid x_{12} at position 12 is any amino acid

and wherein the substitution at position 5 does not result in an A, P or T.

In another preferred embodiment, the above variant of a 3-methylcrotonic acid decarboxylase (MDC) is a variant having an amino acid sequence as shown in SEQ ID NO:1 or a sequence which is derived from SEQ ID NO:1 or an amino acid sequence having at least 60% sequence identity to SEQ ID NO:1 or a sequence which is derived from SEQ ID NO:1, in which one amino acid is substituted, deleted and/or inserted at position 5 in the amino acid motif $K-X_2-G-x_4-X_5-X_6-H-R-X_9-X_{10}-X_{11}-x_{12}-G$ in the amino acid sequence shown in SEQ ID NO:1 or a sequence which is derived from SEQ ID NO:1 or at a position corresponding to this position and wherein said MDC variant has an improved activity in converting 3-methylcrotonic acid into isobutene.

The above variant of a 3-methylcrotonic acid decarboxylase (MDC) having an amino acid sequence as shown in SEQ ID NO:1 or a sequence which is derived from SEQ ID NO:1 is not limited to a specific sequence. A sequence which is derived from SEQ ID NO:1 may be any sequence having the amino acid motif $K-X_2-G-x_4-X_5-X_6-H-R-X_9-X_{10}-X_{11}-x_{12}-G$ and having an activity in converting 3-methylcrotonic acid into isobutene. Examples for sequences which are derived from SEQ ID NO:1, having the amino acid motif $K-X_2-G-x_4-X_5-X_6-H-R-X_9-X_{10}-X_{11}-x_{12}-G$ and having an activity in converting 3-methylcrotonic acid into isobutene, are shown in Table 5.

In a preferred embodiment, the above variant of a 3-methylcrotonic acid decarboxylase (MDC) is a variant wherein an amino acid residue at position 5 in the amino acid motif of an MDC $K-X_2-G-x_4-X_5-X_6-H-R-X_9-X_{10}-X_{11}-x_{12}-G$ is deleted or substituted with an M or F.

In another preferred embodiment, the above variant of a 3-methylcrotonic acid decarboxylase (MDC) is a variant having at least n% sequence identity to an amino acid sequence as shown in the amino acid sequence of a protein having the UniProt accession number selected from the group consisting of the UniProt accession numbers shown in Table 5 with n being an integer between 60 and 100, preferably 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99. As regards the determination of the sequence identity, the same applies as has been set forth above.

The present invention also relates to a method for providing a variant of an MDC wherein said variant shows an improved activity of converting 3-methylcrotonic acid into isobutene, said method comprising the step of effecting one or more changes in

the sequence of the MDC wherein said change(s) is/are effected at one or more amino acid positions selected from the group consisting of the amino acid positions corresponding to positions 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 25, 29, 30, 31, 33, 34, 35, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 89, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 114, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 195, 197, 206, 211, 213, 214, 215, 216, 221, 222, 228, 232, 244, 247, 264, 278, 284, 285, 293, 303, 305, 306, 326, 337, 338, 341, 342, 345, 349, 351, 352, 375, 376, 377, 381, 384, 386, 388, 392, 395, 399, 402, 404, 405, 406, 414, 420, 422, 429, 435, 436, 439, 440, 441, 442, 443, 445, 447, 448, 449, 454, 460, 461, 462, 484, 488, 493, 494, 496, 500, 501, 502, 506, 509, 511 and 512 in the amino acid sequence shown in SEQ ID NO:1. "Corresponding to" means corresponding to any of these positions in a related sequence.

As regards the preferred embodiments of an MDC to be mutated according to such a method, the same applies as has been set forth herein-above.

In one preferred embodiment the MDC from which the MDC variant is derived is an MDC which shows the amino acid sequence as shown in SEQ ID NO:1 or an amino acid sequence having at least 60%, 70%, 80% or 90% sequence identity to SEQ ID NO:1 or any of the preferred degrees of sequence identity as specified herein above.

Moreover, as regards preferred embodiments of the degree of improvement in activity and the changes to be effected, the same applies as described herein above.

The change(s) which is/are effected at any of the above position(s) is/are substitution(s), deletion(s) and/or insertion(s) as defined herein above.

An MDC variant of the present invention can be fused to a homologous or heterologous polypeptide or protein, an enzyme, a substrate or a tag to form a fusion protein. Fusion proteins in accordance with the present invention will have the same improved activity as the MDC variant of the present invention. Polypeptides, enzymes, substrates or tags that can be added to another protein are known in the art. They may be useful for purifying or detecting the proteins of the invention. For instance, tags that can be used for detection and/or purification are e.g. FLAG-tag, His6-tag or a Strep-tag. Alternatively, the protein of the invention can be fused to an enzyme e.g. luciferase, for the detection or localisation of said protein. Other fusion partners include, but are not limited to, bacterial β -galactosidase, trpE, Protein A, β -lactamase, alpha amylase, alcohol dehydrogenase or yeast alpha mating factor. It is also conceivable that the polypeptide, enzyme, substrate or tag is removed from the protein of the invention after e.g. purification. Fusion proteins can typically be made by either recombinant nucleic acid methods or by synthetic polypeptide methods known in art.

The present invention further relates to a nucleic acid molecule encoding an MDC variant of the present invention and to a vector comprising said nucleic acid molecules. Vectors that can be used in accordance with the present invention are known in the art. The vectors can further comprise expression control sequences operably linked to the nucleic acid molecules of the present invention contained in the vectors. These expression control sequences may be suited to ensure transcription and synthesis of a translatable RNA in bacteria or fungi. Expression control sequences can for instance be promoters. Promoters for use in connection with the nucleic acid molecules of the present invention may be homologous or heterologous with regard to its origin and/or with regard to the gene to be expressed. Suitable promoters are for instance promoters which lend themselves to constitutive expression. However, promoters which are only activated at a point in time determined by external influences can also be used. Artificial and/or chemically inducible promoters may be used in this context.

Preferably, the vector of the present invention is an expression vector. Expression vectors have been widely described in the literature. As a rule, they contain not only a selection marker gene and a replication-origin ensuring replication in the host selected, but also a bacterial or viral promoter, and in most cases a termination signal for transcription. Between the promoter and the termination signal there is in general at least one restriction site or a polylinker which enables the insertion of a coding DNA sequence. The DNA sequence naturally controlling the transcription of the corresponding gene can be used as the promoter sequence, if it is active in the selected host organism. However, this sequence can also be exchanged for other promoter sequences. It is possible to use promoters ensuring constitutive expression of the gene and inducible promoters which permit a deliberate control of the expression of the gene. Bacterial and viral promoter sequences possessing these properties are described in detail in the literature. Regulatory sequences for the expression in microorganisms (for instance *E. coli*, *S. cerevisiae*) are sufficiently described in the literature. Promoters permitting a particularly high expression of a downstream sequence are for instance the T7 promoter (Studier et al., Methods in Enzymology 185 (1990), 60-89), lacUV5, trp, trp-lacUV5 (DeBoer et al., in Rodriguez and Chamberlin (Eds), Promoters, Structure and Function; Praeger, New York, (1982), 462-481; DeBoer et al., Proc. Natl. Acad. Sci. USA (1983), 21-25), lp1, rac (Boros et al., Gene 42 (1986), 97-100). Inducible promoters are preferably used for the synthesis of polypeptides. These promoters often lead to higher polypeptide yields than do constitutive promoters. In order to obtain an optimum amount of polypeptide, a two-stage process is often used. First, the host cells are cultured

under optimum conditions up to a relatively high cell density. In the second step, transcription is induced depending on the type of promoter used. In this regard, a tac promoter is particularly suitable which can be induced by lactose or IPTG (=isopropyl- β -D-thiogalactopyranoside) (deBoer et al., Proc. Natl. Acad. Sci. USA 80 (1983), 21-25). Termination signals for transcription are also described in the literature.

In addition, the present invention relates to a host cell comprising the nucleic acid molecule or the vector of the present invention.

In a preferred embodiment, the host cell according to the presenting invention is a microorganism, in particular a bacterium or a fungus. In a more preferred embodiment, the host cell of the present invention is *E. coli*, a bacterium of the genus *Clostridium* or a yeast cell, such as *S. cerevisiae*. In another preferred embodiment the host cell is a plant cell or a non-human animal cell.

The transformation of the host cell with a vector according to the invention can be carried out by standard methods, as for instance described in Sambrook and Russell (2001), Molecular Cloning: A Laboratory Manual, CSH Press, Cold Spring Harbor, NY, USA; Methods in Yeast Genetics, A Laboratory Course Manual, Cold Spring Harbor Laboratory Press, 1990. The host cell is cultured in nutrient media meeting the requirements of the particular host cell used, in particular in respect of the pH value, temperature, salt concentration, aeration, antibiotics, vitamins, trace elements etc.

As mentioned above, the enzymatic conversion of 3-methylcrotonic acid into isobutene utilizing an MDC is preferably performed in the presence of an FMN prenyl transferase and relies on a reaction of two consecutive steps catalyzed by the two enzymes, i.e., the MDC (catalyzing the actual decarboxylation of 3-methylcrotonic acid into isobutene) with an FMN prenyl transferase which provides the modified flavin cofactor. The flavin cofactor may preferably be FMN or FAD. FMN (flavin mononucleotide; also termed riboflavin-5'-phosphate) is a biomolecule produced from riboflavin (vitamin B2) by the enzyme riboflavin kinase and functions as prosthetic group of various reactions. FAD (flavin adenine dinucleotide) is a redox cofactor, more specifically a prosthetic group, involved in several important reactions in metabolism. Thus, in a preferred embodiment, when producing isobutene from 3-methylcrotonic acid comprising the step of incubating an MDC variant of the invention with 3-methylcrotonic acid an FMN prenyl transferase is present which, in a first step, modifies a flavin cofactor (FMN or FAD) into a (modified) flavin-derived cofactor. FMN prenyl transferase prenylates the flavin ring of the flavin cofactor (FMN or FAD) into a

(modified) prenylated flavin cofactor. This reaction is schematically illustrated in Figure 1A.

In a second step, the actual conversion of 3-methylcrotonic acid into isobutene is catalyzed by said MDC variant via a 1,3-dipolar cycloaddition based mechanism wherein said FMN-dependent decarboxylase uses the prenylated flavin cofactor (FMN or FAD) provided by the associated FMN prenyl transferase. This reaction is schematically illustrated in Figure 1B.

Thus, preferably, the host cell of the present invention is a cell which expresses an FMN prenyl transferase capable of modifying a flavin cofactor (FMN or FAD) into a (modified) flavin-derived cofactor. In a preferred embodiment, the host cell is a cell which naturally (endogenously) expresses an FMN prenyl transferase. In another preferred embodiment, the host cell is a cell which recombinantly expresses an FMN prenyl transferase by, e.g., introducing a nucleic acid molecule encoding an FMN prenyl transferase or a vector comprising such a nucleic acid molecule.

In a preferred embodiment, said FMN prenyl transferase which modifies the flavin cofactor (FMN or FAD) into a (modified) flavin-derived cofactor is a phenylacrylic acid decarboxylase (PAD)-type protein, or the closely related prokaryotic enzyme UbiX, an enzyme which is involved in ubiquinone biosynthesis in prokaryotes.

In *Escherichia coli*, the protein UbiX (also termed 3-octaprenyl-4-hydroxybenzoate carboxy-lyase) has been shown to be involved in the third step of ubiquinone biosynthesis.

It catalyses the reaction 3-octaprenyl-4-hydroxybenzoate $\rightleftharpoons$ 2-octaprenylphenol + CO₂.

Thus, in a preferred embodiment, the modification of a flavin cofactor (FMN or FAD) into the corresponding (modified) flavin-derived cofactor is catalyzed by the FMN-containing protein phenylacrylic acid decarboxylase (PAD). The enzymes involved in the modification of the flavin cofactor (FMN or FAD) into the corresponding modified flavin-derived cofactor were initially annotated as decarboxylases (EC 4.1.1.-). Some phenylacrylic acid decarboxylases (PAD) are now annotated as flavin prenyl transferases as EC 2.5.1.-.

In a more preferred embodiment, the conversion of 3-methylcrotonic acid into isobutene makes use of a phenylacrylic acid decarboxylase (PAD)-type protein as the FMN prenyl transferase which modifies a flavin cofactor (FMN or FAD) into the corresponding (modified) flavin-derived cofactor wherein said phenylacrylic acid decarboxylase (PAD)-type protein is derived from *Candida albicans* (Uniprot accession number Q5A8L8), *Aspergillus niger* (Uniprot accession number A3F715),

Saccharomyces cerevisiae (Uniprot accession number P33751) or *Cryptococcus gattii* (Uniprot accession number E6R9Z0).

In a preferred embodiment, the phenylacrylic acid decarboxylase (PAD)-type protein employed in the method of the present invention is a phenylacrylic acid decarboxylase (PAD)-type protein derived from *Candida albicans* (Uniprot accession number Q5A8L8; SEQ ID NO:3), *Aspergillus niger* (Uniprot accession number A3F715; SEQ ID NO:4), *Saccharomyces cerevisiae* (Uniprot accession number P33751; SEQ ID NO:5) or *Cryptococcus gattii* (Uniprot accession number E6R9Z0; SEQ ID NO:6) having the amino acid sequence as shown in SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6, respectively.

In a preferred embodiment of the present invention the phenylacrylic acid decarboxylase (PAD)-type protein is an enzyme comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 3 to 6 or a sequence which is at least n % identical to any of SEQ ID NOs: 3 to 6 with n being an integer between 10 and 100, preferably 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 and wherein the enzyme has the enzymatic activity of modifying a flavin cofactor (FMN or FAD) into the corresponding (modified) flavin-derived cofactor.

As regards the determination of sequence identity, the same applies as has been set forth above.

In another preferred embodiment, the modification of a flavin cofactor (FMN or FAD) into the corresponding (modified) flavin-derived cofactor is catalyzed by the FMN-containing protein 3-octaprenyl-4-hydroxybenzoate carboxy-lyase also termed UbiX (initially annotated EC 4.1.1.-). As mentioned above, the enzymes involved in the modification of the flavin cofactor (FMN or FAD) into the corresponding modified flavin-derived cofactor were initially annotated as decarboxylases. Some phenylacrylic acid decarboxylases (PAD) are now annotated as flavin prenyl transferases as EC 2.5.1.-.

In a more preferred embodiment, the conversion of 3-methylcrotonic acid into isobutene makes use of a 3-octaprenyl-4-hydroxybenzoate carboxy-lyase (also termed UbiX) as the FMN prenyl transferase which modifies the flavin cofactor (FMN or FAD) into the corresponding (modified) flavin-derived cofactor wherein said 3-octaprenyl-4-hydroxybenzoate carboxy-lyase (also termed UbiX) is derived from *Escherichia coli* (Uniprot accession number P0AG03), *Bacillus subtilis* (Uniprot accession, number A0A086WXG4), *Pseudomonas aeruginosa* (Uniprot accession

number A0A072ZCW8) or *Enterobacter* sp. DC4 (Uniprot accession number W7P6B1).

In an even more preferred embodiment, the 3-octaprenyl-4-hydroxybenzoate carboxy-lyase (also termed UbiX) employed in the method of the present invention is a 3-octaprenyl-4-hydroxybenzoate carboxy-lyase (also termed UbiX) derived from *Escherichia coli* (Uniprot accession number P0AG03; SEQ ID NO:2), *Bacillus subtilis* (Uniprot accession, number A0A086WYG4; SEQ ID NO:7), *Pseudomonas aeruginosa* (Uniprot accession number A0A072ZCW8; SEQ ID NO:8) or *Enterobacter* sp. DC4 (Uniprot accession number W7P6B1; SEQ ID NO:9) having the amino acid sequence as shown in SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:8 and SEQ ID NO:9, respectively.

In a preferred embodiment of the present invention the 3-octaprenyl-4-hydroxybenzoate carboxy-lyase is an enzyme comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 7 to 9 or a sequence which is at least n % identical to any of SEQ ID NOs: 2 and 7 to 9 with n being an integer between 10 and 100, preferably 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 and wherein the enzyme has the enzymatic activity of modifying a flavin cofactor (FMN or FAD) into the corresponding (modified) flavin-derived cofactor. As regards the determination of the sequence identity, the same applies as has been set forth above.

The present invention also relates to a method for producing isobutene from 3-methylcrotonic acid comprising the step of incubating an MDC variant of the invention with 3-methylcrotonic acid under conditions allowing said conversion (preferably further in the presence of an FMN prenyl transferase as described above) or comprising the step of culturing a host cell of the present invention expressing an MDC variant (and preferably further expressing an FMN prenyl transferase as described above) in a suitable medium and recovering the produced isobutene.

It is also conceivable in this context that in such a method not only one enzyme according to the present invention is employed but a combination of two or more enzymes.

The present invention also relates to the use of an MDC variant or a host cell of the present invention as described above for the conversion of 3-methylcrotonic acid into isobutene, preferably in the presence of an FMN prenyl transferase or in the presence of a host co-expressing an FMN prenyl transferase as described herein above. Moreover, in a further embodiment, the present invention relates to a method

for producing isobutene from 3-methylcrotonic acid by bringing 3-methylcrotonic acid into contact with the MDC variant of the present invention, preferably in the presence of an FMN prenyl transferase, or with a host cell comprising a nucleic acid molecule encoding the MDC variant of the present invention, wherein said host cell preferably expresses an FMN prenyl transferase. Thus, in a preferred embodiment, the present invention relates to a method for converting 3-methylcrotonic acid into isobutene comprising the steps of: (i) culturing the above-described host cell of the invention in a suitable medium; and (ii) achieving the production of isobutene from 3-methylcrotonic acid.

Thus, in a preferred embodiment, the present invention relates to methods and uses utilizing a host cell of the present invention which expresses an MDC variant of the present invention and, preferably, further expressing an FMN prenyl transferase as described herein above.

In another preferred embodiment, such a host cell is an organism which is capable of producing 3-methylcrotonic acid.

In another preferred embodiment, the method according to the invention is carried out in culture, in the presence of an organism, preferably a microorganism, producing an enzyme variant of the present invention and, preferably, also producing an FMN prenyl transferase. In such an embodiment of the invention, an organism, preferably a microorganism, that produces an enzyme of the present invention and, preferably, also producing an FMN prenyl transferase, is used. In a preferred embodiment, the (micro)organism is recombinant in that the enzyme produced by the host is heterologous relative to the production host. The method can thus be carried out directly in the culture medium, without the need to separate or purify the enzymes. In an especially advantageous manner, a (micro)organism is used having the natural or artificial property of endogenously producing 3-methylcrotonic acid so as to produce isobutene directly from the substrate already present in the culture in solution.

In connection with the above described methods and uses, the microorganisms are cultivated under suitable culture conditions allowing the occurrence of the enzymatic reaction of the MDC variants of the present invention (and, preferably, also the FMN prenyl transferases as described above). The specific culture conditions depend on the specific microorganism employed but are well known to the person skilled in the art. The culture conditions are generally chosen in such a manner that they allow the

expression of the genes encoding the MDC variant of the present invention (and, preferably, also an FMN prenyl transferase as described above). Various methods are known to the person skilled in the art in order to improve and fine-tune the expression of certain genes at certain stages of the culture such as induction of gene expression by chemical inducers or by a temperature shift.

In another embodiment, the above described methods of the invention comprise the step of providing the organism, preferably the microorganism carrying the respective enzyme activity or activities in the form of a (cell) culture, preferably in the form of a liquid cell culture, a subsequent step of cultivating the organism, preferably the microorganism in a fermenter (often also referred to a bioreactor) under suitable conditions allowing the expression of the respective enzyme and further comprising the step of effecting an enzymatic conversion of a method of the invention as described herein above. Suitable fermenter or bioreactor devices and fermentation conditions are known to the person skilled in the art. A bioreactor or a fermenter refers to any manufactured or engineered device or system known in the art that supports a biologically active environment. Thus, a bioreactor or a fermenter may be a vessel in which a chemical/biochemical process like the method of the present invention is carried out which involves organisms, preferably microorganisms and/or biochemically active substances, i.e., the enzyme(s) described above derived from such organisms or organisms harboring the above described enzyme(s). In a bioreactor or a fermenter, this process can either be aerobic or anaerobic. These bioreactors are commonly cylindrical, and may range in size from litres to hundreds of cubic meters, and are often made of stainless steel. In this respect, without being bound by theory, the fermenter or bioreactor may be designed in a way that it is suitable to cultivate the organisms, preferably microorganisms, in, e.g., a batch-culture, feed-batch-culture, perfusion culture or chemostate-culture, all of which are generally known in the art.

The culture medium can be any culture medium suitable for cultivating the respective organism or microorganism.

In yet a further embodiment, the method according to the invention can be carried out *in vitro*, e.g. in the presence of isolated enzyme or of cell lysates comprising the enzyme or partially purified enzyme preparations comprising the MDC variant of the present invention (and, preferably, also an FMN prenyl transferase as described above). *In vitro* preferably means in a cell-free system.

In one embodiment, the enzyme(s) employed in the method is (are) used in purified form. However, such a method may be costly, since enzyme and substrate production and purification costs are high.

Thus, in another preferred embodiment, the enzymes employed in the method are present in the reaction as a non-purified extract, or else in the form of non-lysed bacteria, so as to economize on protein purification costs. However, the costs associated with such a method may still be quite high due to the costs of producing and purifying the substrates.

In an *in vitro* reaction the enzymes, native or recombinant, purified or not, are incubated in the presence of the substrate in physicochemical conditions allowing the enzymes to be active, and the incubation is allowed to proceed for a sufficient period of time allowing production of the desired product as described above. At the end of the incubation, one optionally measures the presence of isobutene by using any detection system known to one of skill in the art such as gas chromatography or colorimetric tests for measuring the formation of isobutene.

In a particularly preferred embodiment of the invention the method is carried out *in vitro* and the enzyme is immobilized. Means and methods for immobilizing enzymes on different supports are well-known to the person skilled in the art.

The method according to the invention furthermore comprises the step of collecting gaseous products, i.e. isobutene, degassing out of the reaction, i.e. recovering the product which degasses, e.g., out of the culture. Thus, in a preferred embodiment, the method is carried out in the presence of a system for collecting isobutene under gaseous form during the reaction.

As a matter of fact, isobutene adopts the gaseous state at room temperature and atmospheric pressure. Moreover, isobutene also adopts the gaseous state under culture conditions at 37 °C. The method according to the invention therefore does not require extraction of isobutene from the liquid culture medium, a step which is always very costly when performed at industrial scale. The evacuation and storage of gaseous hydrocarbons, in particular of isobutene, and their possible subsequent physical separation and chemical conversion can be performed according to any method known to one of skill in the art.

Finally, the present invention relates to a composition comprising a variant of an MDC of the present invention, a nucleic acid molecule of the present invention, a vector of the present invention or a host cell of the present invention. As regards the variant of an MDC, the nucleic acid molecule, the vector or the host cell, the same

applies as has been set forth above in connection with the methods according to the present invention.

In this specification, a number of documents including patent applications are cited. The disclosure of these documents, while not considered relevant for the patentability of this invention, is herewith incorporated by reference in its entirety. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

Figure 1A: shows a schematic reaction of the enzymatic prenylation of a flavin mononucleotide (FMN) into the corresponding modified (prenylated) flavin cofactor.

Figure 1B: Schematic reaction of the enzymatic conversion of 3-methylcrotonic acid into isobutene.

Figure 2: Multiple Sequence Alignment of 19 protein homologues of the *Hypocrea atroviridis* Ferulic acid decarboxylase 1 (G9NLP8), focussing on the K401-G413 segment of G9NLP8.

Figure 3: Isobutene (IBN) production from 10 mM 3-methylcrotonic acid of homologues MDC and their T->M variants at different temperatures (30, 40, 50 and 60°C).

The invention will now be described by reference to the following examples which are merely illustrative and are not to be construed as a limitation of the scope of the present invention.

Examples

Example 1: Directed Evolution Strategy

The enzyme *Hypocrea atroviridis* Ferulic acid decarboxylase 1 (SEQ ID NO:1) is capable of catalysing, amongst other reactions, the decarboxylation of 3-methylcrotonic acid (3MC) into isobutene (IBN). A directed evolution approach was

used in order to specifically improve the catalytic efficiency of this reaction. This approach consisted in (1) the design of assay systems to test the activity of enzyme variants, (2) the generation of collections of single point or multiple mutants for *Hypocrea atroviridis* Ferulic acid decarboxylase 1, and (3) the use of the activity assays to screen the collection of mutants in order to identify variants with improved activity compared to the activity of the wild type *Hypocrea atroviridis* Ferulic acid decarboxylase 1.

This approach led to the identification and characterization of a collection of mutants with increased activity compared to the wild type enzyme.

Example 2: Construction of *Hypocrea atroviridis* Ferulic acid decarboxylase 1 enzyme mutants

The polynucleotide sequences coding for the different mutants identified during the evolution of the *Hypocrea atroviridis* Ferulic acid decarboxylase 1 enzyme were generated using a range of standard molecular biology techniques. All these techniques used a codon-optimised polynucleotide sequence for expression in *Escherichia coli* as template. The sequence optimisation has been done by Geneart using their GeneOptimizer software.

Different PCR-based techniques known in the art were used for the construction of single-point mutants. For the generation of enzyme variants bearing multiple mutations (at least two mutations), either PCR-based techniques or other methods known in the art were used to introduce these mutations.

Following mutagenesis, the mutated polynucleotide sequence was inserted into a pETDuet™-1 co-expression vector (Novagen) (used for recombinant protein production in *E. coli* and screening) in addition to the cDNA of the Flavin prenyltransferase UbiX protein from *E. coli* either using standard ligase-based subcloning techniques, whole plasmid extension by PCR or ligase-independent cloning techniques.

Example 3: Selection of the enzyme mutants with increased activity

Two different screening methods were developed and used during the evolution of the *Hypocrea atroviridis* Ferulic acid decarboxylase 1 enzyme.

1.) In vivo assay in 384-well microplates based on exogenous 3MC (IN VIVO 1)

This assay is based on the use of a bacterial strain (BL21(DE3), Novagen) transformed with the above expression vector that contain the above-described coding sequences leading to the production of the last two enzymes involved in the metabolic pathway converting 3MC into isobutene, namely the *Hypocrea atroviridis* Ferulic acid decarboxylase 1 variants and the Flavin prenyltransferase UbiX protein from *E. coli*. This strain is first plated out onto LB-agar plates supplemented with the appropriate antibiotic. Cells were grown overnight at 30°C until individual colonies reach the desired size. Single colonies were then picked and individually transferred into 50µL of liquid LB medium supplemented with the appropriate antibiotic. Cell growth is carried out with shaking for 20 hours at 30°C. The LB cultures were used to inoculate 300 µL in 384 deepwell microplates of auto-induction medium (Studier FW, *Prat.Exp.Pur.* 41, (2005), 207-234) supplemented with the appropriate antibiotic and grown in a shaking incubator set at 700rpm and 85% humidity for 24h at 30°C in order to produce the two recombinant enzymes. The cell pellet containing these two overexpressed recombinant enzymes is then resuspended in 40 µL of minimum medium (pH 7.5, Phosphate 100 mM, Glucose 10g.L⁻¹, MgSO₄ 1mM) supplemented with 10 mM 3MC and incubated for a further 2 or 4 hours in a shaking incubator at 30°C, 700 rpm. During this step, the *Hypocrea atroviridis* Ferulic acid decarboxylase 1 variants catalyse the decarboxylation of 3MC into IBN. After 5 min inactivation at 80°C, the IBN produced is quantified by gas chromatography as described in the following. 100 µL of headspace gases from each enzymatic reaction are injected in a Brucker GC-450 system equipped with a Flame Ionization Detector (FID). Compounds present in samples were separated by chromatography using a RTX-1 columns at 100°C with a 1 mL.min⁻¹ constant flow of nitrogen as carrier gas. Upon injection, peak areas of isobutene were calculated.

2.) In vivo assay in 96-well microplates based on exogenous 3MC (IN VIVO 2)

This assay is based on the use of a bacterial strain (BL21(DE3), Novagen) transformed with the above expression vector that contains the coding sequences as described above, leading to the production of the last two enzymes involved in the metabolic pathway converting 3MC to isobutene; namely the *Hypocrea atroviridis* Ferulic acid decarboxylase 1 variants and the Flavin prenyltransferase UbiX protein from *E. coli*. This strain is first plated out onto LB-agar plates supplemented with the appropriate antibiotic. Cells were grown overnight at 30°C until individual colonies reach the desired size. Single colonies were then picked and individually transferred into 500µL of liquid LB medium supplemented with the appropriate antibiotic. Cell growth is carried out with shaking for 20 hours at 30°C or 32°C. The LB cultures were

used to inoculate 1 mL in 96 deepwell microplates of auto-induction medium (Studier FW, *Prat.Exp.Pur.* 41, (2005), 207-234) supplemented with the appropriate antibiotic and grown in a shaking incubator set at 700rpm and 85% humidity for 24h at 30°C or 32°C in order to produce the two recombinant enzymes. The cell pellet containing these two overexpressed recombinant enzymes is then resuspended in 400 µL of minimum medium (pH 7.5, Phosphate 100 mM, Glucose 10g.L⁻¹, MgSO₄ 1mM) supplemented with 10 mM 3MC and incubated for a further 2 or 4 hours in a shaking incubator at 30°C or 36°C, 700 rpm. During this step, the *Hypocrea atroviridis* Ferulic acid decarboxylase 1 variants catalyse the decarboxylation of 3MC into IBN. After 5 min inactivation at 80°C, the IBN produced is quantified by gas chromatography as described in the following. 100 µL of headspace gases from each enzymatic reaction are injected in a Brucker GC-450 system equipped with a Flame Ionization Detector (FID). Compounds present in samples were separated by chromatography using a RTX-1 columns at 100°C with a 1 mL.min⁻¹ constant flow of nitrogen as carrier gas. Upon injection, peak areas of isobutene were calculated.

Example 4: Identification of variants of *Hypocrea atroviridis* Ferulic acid decarboxylase 1 with further increased activity for the reaction of conversion of 3-methylcrotonic acid into isobutene

A collection of mutants has been created by mutagenesis, using the *Hypocrea atroviridis* Ferulic acid decarboxylase 1 (SEQ ID NO:1) as template. Several variants with an enhanced activity in converting 3-methylcrotonic acid into isobutene have been identified through *in vivo* screening assays as described above. A first round of screening with 59508 clones has been performed using the above IN VIVO 1 assay. The best 927 clones were then tested in 12-replicate using the same protocol. The plasmids of the best 95 variants were individually extracted, transformed into fresh competent BL21(DE3) cells and then tested in 8-replicate assays according to the above-described IN VIVO 2 assay.

The list of improved variants is presented in the following **Table 1** and the list of the individual positions presenting an increase in activity is shown in **Table 2**. The improvement factor shown in the below Table 1 reflects the average value of 8 replicates of the relative quantity of isobutene which has been produced and measured according to the above-described IN VIVO 2 assay compared to the wild type enzyme.

Table 1: List of *Hypocrea atroviridis* Ferulic acid decarboxylase 1 variants presenting an increase in isobutene production from 3-methylcrotonic acid

Mutations	Improvement Factor / WT
T405M	7.9
T405F	4.7
S2Q	4.4
S2A	4.3
L195C	4.3
L449I	4.3
S2K	4.2
S2L	4.1
S2V	4.0
S3A	3.9
S3Y	3.8
S2F	3.7
S3K L511M	3.7
T4N F91L	3.5
S2N	3.5
T4E	3.5
S3K M284Y	3.5
S3W	3.2
S3G	3.2
M293L	3.2
S3P	3.1
T4M	3.1
S3E	3.0
T405L	2.9
S2D E89S	2.8
T4L	2.7
V40M	2.6
D35M	2.5
K422M	2.4

Q448W	2.3
L195I	2.3
A285L	2.3
L195W	2.2
V34A	2.2
D12S	2.1
L500A	2.1
D351R	2.1
V34I	2.1
Q214T	2.1
E9Y	2.0
L195M	2.0
F447W	2.0
L114S	2.0
P13N	1.9
E9H	1.9
F11P	1.8
D43R	1.8
D420L	1.8
P13I	1.8
L195Y	1.8
V439L	1.8
I337M	1.8
L506I	1.8
F11L	1.7
P13Y	1.7
P120S	1.7
T429S	1.7
T436N	1.7
G435M	1.7
L195V	1.7
A149V	1.6

F447Y	1.6
P13S	1.6
Q29N	1.6
A10H	1.6
N31G	1.6
Q214A	1.5
V40I	1.5
L195F	1.5
N31E N501E	1.5
E25N	1.5
T429A	1.4
N501K	1.4
E9P	1.4
I197F	1.4
Q448S	1.4
D35T	1.4
A146S	1.4
D442T	1.4
P13H	1.3
Q214V	1.3
V445P	1.3
V445E	1.3
L33I	1.3
A381R	1.3
L221C	1.3
L449M	1.2
T405Q	1.2
T405P	1.2
T376I A388E	1.2
D35S	1.2
L449V	1.2
Q29S	1.2

N141D	1.2
F441Y	1.2

Table 2: List of the positions modified in the variants of *Hypocrea atroviridis* Ferulic acid decarboxylase 1 with increased activity

Position	Wild-Type Amino Acid	Mutations
2	S	A, D, F, K, L, N, Q, V
3	S	A, E, G, K, P, W, Y,
4	T	E, L, M, N
9	E	H, P, Y
10	A	H
11	F	L, P
12	D	S
13	P	H, I, N, S, Y
25	E	N
29	Q	N, S
31	N	E, G
33	L	I
34	V	A, I
35	D	M, S, T
40	V	I, M
43	D	R
89	E	S
91	F	L
114	L	S
120	P	S
141	N	D
146	A	S
149	A	V

		C, F, I, M, V, W, Y
195	L	
197	I	F
214	Q	A, T, V
221	L	C
284	M	Y
285	A	L
293	M	L
337	I	M
351	D	R
376	T	I
381	A	R
388	A	E
405	T	F, L, M, P, Q
420	D	L
422	K	M
429	T	A, S
435	G	M
436	T	N
439	V	L
441	F	Y
442	D	T
445	V	E, P
447	F	W, Y
448	Q	S, W
449	L	I, M, V
500	L	A
501	N	E, K
506	L	I
511	L	M

Example 5: *In vitro* activities of the *Hypocrea atroviridis* Ferulic acid 1 decarboxylase WT and T405M mutant

Gene synthesis, cloning, expression and purification of Ferulic acid decarboxylases

The pETDuet™-1 co-expression vectors encoding *Hypocrea atroviridis* Ferulic acid 1 decarboxylase WT or T405M variant and the Flavin prenyltransferase UbiX protein from *E. coli* were obtained according to the procedure described in Example 2 and 3. The provided vector contained a stretch of 6 histidine codons after the methionine initiation codon of the ferulic acid decarboxylases in order to specifically purify the ferulic acid decarboxylase.

Competent *E. coli* BL21 (DE3) cells (Novagen) were transformed with these vectors according to standard heat shock procedures. The transformed cells were grown with shaking (160 rpm) using ZYM-5052 auto-induction medium (Studier FW, Prot. Exp. Pur. 41, (2005), 207-234) at 30°C during 24 h. The cells were collected by centrifugation at 4°C, 10,000 rpm for 20 min and the pellets were stored at -80°C. Pellets from 500 ml of culture cells were thawed on ice and resuspended in 15 ml of 50 mM potassium phosphate buffer containing 200 mM NaCl, 10 mM MgCl₂, 10 mM imidazole and 1 mM DTT. Twenty microliters of lysonase (Novagen) were added. Cells were incubated 10 minutes at room temperature and then returned to ice for 20 minutes. Cell lysis was completed by sonication for 2 x 15 seconds.

The bacterial extracts were then clarified by centrifugation at 4°C, 4000 rpm for 40 min. The clarified bacterial lysates were loaded onto a PROTINO-2000 Ni-TED column (Macherey-Nagel) allowing adsorption of 6-His tagged proteins. Columns were washed and the enzymes of interest were eluted with 6 ml of 50 mM potassium phosphate buffer containing 250 mM imidazole. Eluates were then concentrated, desalted on a Amicon Ultra-4 10 kDa filter unit (Millipore) and enzymes were resuspended in 50 mM potassium phosphate buffer containing 1 mM DTT and 20 mM NaCl. The purity of Ferulic acid decarboxylases thus purified varied from 80% to 90% as estimated by SDS-PAGE analysis. Protein concentrations were determined by direct UV 280 nm measurement on the NanoDrop 1000 spectrophotometer (Thermo Scientific) or by a Bradford assay (BioRad).

Production of Flavin prenyltransferases

In order to perform enzymatic assays, the Flavin prenyltransferase UbiX protein from *E. coli* was also produced separately. Vector pCAN containing the gene coding for the Flavin prenyltransferase UbiX protein from *E. coli* was purchased from NAIST (Nara Institute of Science and Technology, Japan, ASKA collection).

Competent *E. coli* BL21 (DE3) cells (Novagen) were transformed with this vector according to standard heat shock procedures. The transformed cells were grown with shaking (160 rpm) using ZYM-5052 auto-induction medium (Studier FW, Prot. Exp. Pur. 41, (2005), 207-234) at 30°C during 24 h. The cells were collected by centrifugation at 4°C, 10,000 rpm for 20 min and the pellets were stored at -80°C. Pellets from 500 ml of culture cells were thawed on ice and resuspended in 15 ml of 50 mM potassium phosphate buffer containing 200 mM NaCl, 10 mM MgCl₂, 10 mM imidazole and 1 mM DTT. Twenty microliters of lysonase (Novagen) were added. Cells were incubated 10 minutes at room temperature and then returned to ice for 20 minutes. Cell lysis was completed by sonication for 2 x 15 seconds. The cellular lysate containing the UbiX protein was kept on ice.

Enzymatic assay

Enzymatic assays were performed with Ferulic acid decarboxylase purified as described above, supplemented with a fresh cellular lysate containing the UbiX protein (see above).

0.5 M stock solution of 3-methylcrotonic acid was prepared in water and adjusted to pH 7.0 with 10 M solution of NaOH. Enzymatic assays were carried out in 2 ml glass vials (Interchim) under the following conditions: 50 mM potassium phosphate pH 7.5; 20 mM NaCl; 3 mM MgCl₂; 5 mM DTT; 64 mM 3-methylcrotonic acid; 0.5 mg/ml purified of Ferulic acid decarboxylase (FDC) WT or T405M variant; 100 µl of the lysate contained the Flavin prenyltransferase UbiX protein; total volume of the assays were 300 µl.

The vials were sealed and incubated for 60 minutes at 30°C. A control without Ferulic acid decarboxylase was performed in parallel. The assays were stopped by incubating for 2 minute at 80°C and the isobutene formed in the reaction headspace was analysed by Gas Chromatography (GC) equipped with a Flame Ionization Detector (FID). For the GC headspace analysis, one ml of the headspace gas was separated in a Bruker GC-450 system equipped with a GS-alumina column (30 m x 0.53 mm) (Agilent) using isothermal mode at 130°C. Nitrogen was used as carrier gas with a flow rate of 6 ml/min. The enzymatic reaction product was identified by comparison with an isobutene standard. Under these GC conditions, the retention time of isobutene was 2.42 min.

Results

Under these conditions, the T405M variant of *Hypocrea atroviridis* Ferulic acid decarboxylase 1 is about 7 time more efficient than the WT corresponding enzyme for the conversion of 3-methylcrotonic acid into isobutene; se **Table 3**.

Table 3

	Isobutene peak area, arbitrary unit
FDC WT	1680
FDC T405M	11550
without FDC	2

Example 6: Identification of a signature sequence around position 405 of *Hypocrea atroviridis* Ferulic acid decarboxylase 1 (SEQ ID NO:1)

The two variants of *Hypocrea atroviridis* Ferulic acid decarboxylase 1 (SEQ ID NO:1) having the highest increase in activity harbor a mutation at position 405.

Hypocrea atroviridis belongs to the *Pezizomycotina* subphylum of the *Ascomycota* phylum. Thus, the *Hypocrea atroviridis* Ferulic acid decarboxylase has been compared with 1 to 19 homologue proteins belonging to the *Pezizomycotina* subphylum (Table 4) which are capable of catalyzing the conversion of 3-methylcrotonic acid into isobutene.

These sequences have been compared in a multiple-sequence alignment using the T-Coffee software in “accurate” mode, combining sequence, structure and profile alignment (Notredame et al., JMB 302 (2000), 205-217). From this alignment, a conserved region around the T405 position of the *Hypocrea atroviridis* Ferulic acid decarboxylase 1 has been derived (K401-G413); (Figure 2).

This block of aligned sequences was used to derive a pattern with the PRATT software (Jonassen et al., Protein Science 4(8) (1995), 1587-1595):

K-[APV]-G-x-[APT]-[FIM]-H-R-[IL]-[AILV]-[ILV]-x-G where x represents any amino acid and [XYZ] represents either amino acid X or Y or Z.

This motif was then fed into the ScanProsite webtool (<http://prosite.expasy.org/scanprosite/>) to scan the UniProtKB database (Swiss-Prot including splice variants and TrEMBL). A total of 107 different proteins was found, including the original 20 from which the motif was derived (Table 5).

Among these 107 proteins, 3 are annotated as 3-octaprenyl-4-hydroxybenzoate carboxylase, one as Phenolic acid decarboxylase, one as UbiD-domain-containing protein, 3 as Uncharacterized protein and 99 as Ferulic acid decarboxylase 1. Considering that these annotations represent the decarboxylation of aromatic acids, it is assumed that this motif is representative for the UbiD family.

Moreover, among these 107 proteins, 105 belong to the *Dikarya* sub-kingdom while 19 of these belong to the *Filobasidiella/Cryptococcus neoformans* species complex of the *Agaricomycotina* subphylum, 85 to the *Pezizomycotina* subphylum,

one to the *Saccharomycotina* subphylum and two to the *Phytophthora* genus of the *Peronosporales* order.

Therefore, it is assumed that this motif is not only representative for the UbiD family of the *Pezizomycotina* subphylum from which it was derived, but also for the UbiD family of different fungi and fungus-like eukaryotic microorganisms.

Table 4: List of 19 proteins homologues to *Hypocrea atroviridis* Ferulic acid decarboxylase 1 (G9NLP8) presenting a 3-methylcrotonic acid decarboxylase activity

Entry (UniProt)	Entry name	Protein names	Gene names	Organism	Length
A0A094IED9	A0A094IED9_9PEZI	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 V502_01403	<i>Pseudogymnoascus</i> sp. VKM F-4520 (FW-2644)	589
W9WWR1	W9WWR1_9EURO	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 A105_04852	<i>Cladophialophora psammophila</i> CBS 110553	503
A0A0D2IKD5	A0A0D2IKD5_XYLB A	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 Z519_02676	<i>Cladophialophora bantiana</i> CBS 173.52	503
A0A0D2AQI6	A0A0D2AQI6_9EUR O	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 PV07_07106	<i>Cladophialophora immunda</i>	505
A0A0D2DPQ1	A0A0D2DPQ1_9EU RO	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 PV04_09173	<i>Capronia semiimmersa</i>	499
W9YNA8	W9YNA8_9EURO	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 A101_03830	<i>Capronia coronata</i> CBS 617.96	498
M7THT1	M7THT1_BOTF1	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 BcDW1_8299	<i>Botryotinia fuckelliana</i> (strain BcDW1) (Noble rot fungus) (<i>Botrytis cinerea</i>)	513
M3DF95	M3DF95_SPHMS	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 SEPMUDRAFT_154815	<i>Sphaerulina musiva</i> (strain SO2202) (Poplar stem canker fungus) (<i>Septoria musiva</i>)	508
FOX198	FOX198_GROCL	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 CMQ_6352	<i>Grosmannia clavigera</i> (strain kw1407 / UAMH 11150) (Blue stain fungus) (<i>Graphiocladiella clavigera</i>)	500
W9LTH3	W9LTH3_FUSOX	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 FOWG_11397	<i>Fusarium oxysporum</i> f. sp. lycopersici MN25	503
A2QHE5	FDC1_ASPNC	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	fdc1 An03g06590	<i>Aspergillus niger</i> (strain CBS 513.88 / FGSC A1513)	500
A0A0G4P429	A0A0G4P429_PENC A	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 PCAMFM013_S005g000 265	<i>Penicillium camemberti</i> FM 013	500
W6QKP7	W6QKP7_PENRF	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 PROQFM164_S05g0008 53	<i>Penicillium roqueforti</i> (strain FM164)	498
A1DCG7	A1DCG7_NEOFI	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 NFIA_026010	<i>Neosartorya fischeri</i> (strain ATCC 1020 / DSM 3700 / FGSC A1164 /	505

A0A0F0IHE5				NRRL 181) (<i>Aspergillus fischerianus</i>)	
	A0A0F0IHE5_ASPP U	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 P875_00128011	<i>Aspergillus parasiticus</i> (strain ATCC 56775 / NRRL 5862 / SRRC 143 / SU-1)	503
G9MUK3	G9MUK3_HYPVG	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 TRIVIDRAFT_53354	<i>Hypocrea virens</i> (strain Gv29-8 / FGSC 10586) (<i>Gliocladium virens</i>) (<i>Trichoderma virens</i>)	507
G9NTM9	G9NTM9_HYPAI	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 TRIATDRAFT_299540	<i>Hypocrea atroviridis</i> (strain ATCC 20476 / IMI 206040) (<i>Trichoderma atroviride</i>)	510
G9NLP8	G9NLP8_HYPAI	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 TRIATDRAFT_53567	<i>Hypocrea atroviridis</i> (strain ATCC 20476 / IMI 206040) (<i>Trichoderma atroviride</i>)	512
G9MXT8	G9MXT8_HYPVG	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 TRIVIDRAFT_69398	<i>Hypocrea virens</i> (strain Gv29-8 / FGSC 10586) (<i>Gliocladium virens</i>) (<i>Trichoderma virens</i>)	511
A0A0G0A274	A0A0G0A274_TRIH A	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 THAR02_01458	<i>Trichoderma harzianum</i> (<i>Hypocrea lixii</i>)	511

Table 5: List of the 107 proteins found in the UniProtKB database through ScanProsite, with the K-[APV]-G-x-[APT]-[FIM]-H-R-[IL]-[AILV]-[ILV]-x-G motif.

Entry (UniProt)	Entry name	Protein names	Gene names	Organism	Length
A0A0B4FU01	A0A0B4FU01_9HYP O	3-octaprenyl-4-hydroxybenzoate carboxy-lyase (Fragment)	MBR_10525	<i>Metarhizium brunneum</i> ARSEF 3297	500
A0A0B4ENF4	A0A0B4ENF4_META N	3-octaprenyl-4-hydroxybenzoate carboxy-lyase (Fragment)	MAN_07756	<i>Metarhizium anisopliae</i> ARSEF 549	500
A0A0B8N4Y5	A0A0B8N4Y5_9EUR O	3-octaprenyl-4-hydroxybenzoate carboxylyase	TCE0_047r17842	<i>Talaromyces cellulolyticus</i>	130
A2QHE5	FDC1_ASPNC	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	fdc1 An03g06590	<i>Aspergillus niger</i> (strain CBS 513.88 / FGSC A1513)	500
A0A0A2J5F4	A0A0A2J5F4_PENE N	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 PEX1_019400 PEX2_105470 PEXP_077520	<i>Penicillium expansum</i> (Blue mold rot fungus)	500
A0A0D2YAR9	A0A0D2YAR9_FUSO 4	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 FOXG_13395	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (strain 4287 / CBS 123668 / FGSC 9935 / NRRL 34936) (<i>Fusarium vascular wilt of tomato</i>)	503
A0A0J0DBQ6	A0A0J0DBQ6_GIBF U	Ferulic acid decarboxylase 1 (EC 4.1.1.102) (Phenacrylate decarboxylase)	FDC1 LW94_13187 Y057_969	<i>Gibberella fujikuroi</i> (<i>Bakanae</i> and foot rot disease fungus) (<i>Fusarium fujikuroi</i>)	503

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 177

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 177

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

CLAIMS

1. A variant of a 3-methylcrotonic acid decarboxylase (MDC) showing an improved activity in converting 3-methylcrotonic acid into isobutene over the corresponding MDC from which it is derived and having an amino acid sequence as shown in SEQ ID NO:1 or an amino acid sequence having at least 73% sequence identity to SEQ ID NO:1, in which one or more amino acid residues at a position selected from the group consisting of positions 405, 2, 12, 13, 29, 31, 33, 35, 89, 114, 195, 197, 221, 293, 337, 351, 376, 381, 388, 420, 422, 435, 436, 439, 441, 447, 449, 500, 506 and 511 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to any of these positions, are substituted with another amino acid residue or deleted or wherein an insertion has been effected at one or more of these positions.
2. The MDC variant of claim 1, wherein
 - (1) an amino acid residue at position 2 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, aspartic acid, phenylalanine, lysine, leucine, asparagine, glutamine or valine; and/or
 - (2) an amino acid residue at position 12 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine, alanine or asparagine; and/or
 - (3) an amino acid residue at position 13 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, isoleucine, asparagine, serine, valine or tyrosine; and/or
 - (4) an amino acid residue at position 29 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine, histidine or serine; and/or
 - (5) an amino acid residue at position 31 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid or glycine; and/or

- (6) an amino acid residue at position 33 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (7) an amino acid residue at position 35 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine, serine or threonine; and/or
- (8) an amino acid residue at position 89 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine or phenylalanine; and/or
- (9) an amino acid residue at position 114 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (10) an amino acid residue at position 195 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine, cysteine, phenylalanine, isoleucine, valine, tryptophan or tyrosine; and/or
- (11) an amino acid residue at position 197 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine or methionine; and/or
- (12) an amino acid residue at position 221 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (13) an amino acid residue at position 293 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (14) an amino acid residue at position 337 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine or leucine; and/or
- (15) an amino acid residue at position 351 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine, asparagine, alanine, valine or glycine; and/or
- (16) an amino acid residue at position 376 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (17) an amino acid residue at position 381 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or

- (18) an amino acid residue at position 388 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid; and/or
- (19) an amino acid residue at position 405 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, leucine, methionine, proline or glutamine; and/or
- (20) an amino acid residue at position 420 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (21) an amino acid residue at position 422 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine; and/or
- (22) an amino acid residue at position 435 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine; and/or
- (23) an amino acid residue at position 436 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (24) an amino acid residue at position 439 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (25) an amino acid residue at position 441 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine; and/or
- (26) an amino acid residue at position 447 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tryptophan, methionine or tyrosine; and/or
- (27) an amino acid residue at position 449 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, methionine or valine; and/or
- (28) an amino acid residue at position 500 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or
- (29) an amino acid residue at position 506 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or tyrosine; and/or

- (30) an amino acid residue at position 511 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine or isoleucine.
3. The MDC variant of claim 1 or 2, wherein said variant furthermore shows at least one modification at positions 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15, 25, 30, 34, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 206, 211, 213, 214, 215, 216, 222, 228, 232, 244, 247, 264, 278, 284, 285, 303, 305, 306, 326, 338, 341, 342, 345, 349, 352, 375, 377, 384, 386, 392, 395, 399, 402, 404, 406, 414, 429, 440, 442, 443, 445, 448, 454, 460, 461, 462, 484, 488, 493, 494, 496, 501, 502, 509 and 512 in the amino acid sequence shown in SEQ ID NO:1.
4. The MDC variant of any one of claims 1 to 3, wherein said variant furthermore shows at least one modification at positions 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15, 25, 30, 34, 40, 43, 57, 60, 65, 67, 69, 70, 71, 72, 80, 84, 85, 86, 87, 90, 91, 99, 101, 102, 103, 105, 106, 108, 111, 117, 119, 120, 126, 132, 141, 146, 149, 154, 159, 160, 162, 175, 176, 187, 189, 193, 206, 211, 213, 214, 215, 216, 222, 228, 232, 244, 247, 264, 278, 284, 285, 303, 305, 306, 326, 338, 341, 342, 345, 349, 352, 375, 377, 384, 386, 392, 395, 399, 402, 404, 406, 414, 429, 440, 442, 443, 445, 448, 454, 460, 461, 462, 484, 488, 493, 494, 496, 501, 502, 509 and 512 in the amino acid sequence shown in SEQ ID NO:1 is an MDC variant, wherein
- (1) an amino acid residue at position 3 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, glutamic acid, glycine, lysine, proline, tryptophan, cysteine, aspartic acid or tyrosine; and/or
 - (2) an amino acid residue at position 4 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid, leucine, methionine, alanine, serine or asparagine; and/or
 - (3) an amino acid residue at position 5 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
 - (4) an amino acid residue at position 6 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline; and/or

- (5) an amino acid residue at position 7 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (6) an amino acid residue at position 8 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (7) an amino acid residue at position 9 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, proline or tyrosine; and/or
- (8) an amino acid residue at position 10 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, phenylalanine, lysine, proline, threonine or leucine; and/or
- (9) an amino acid residue at position 11 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine, tyrosine or proline; and/or
- (10) an amino acid residue at position 14 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartic acid; and/or
- (11) an amino acid residue at position 15 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (12) an amino acid residue at position 25 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine, serine or tryptophan; and/or
- (13) an amino acid residue at position 30 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine, histidine or arginine; and/or
- (14) an amino acid residue at position 34 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or isoleucine; and/or
- (15) an amino acid residue at position 40 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or methionine; and/or
- (16) an amino acid residue at position 43 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or

- (17) an amino acid residue at position 57 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (18) an amino acid residue at position 60 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or
- (19) an amino acid residue at position 65 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine or tryptophan; and/or
- (20) an amino acid residue at position 67 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine or valine; and/or
- (21) an amino acid residue at position 69 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (22) an amino acid residue at position 70 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or leucine; and/or
- (23) an amino acid residue at position 71 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or
- (24) an amino acid residue at position 72 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
- (25) an amino acid residue at position 80 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (26) an amino acid residue at position 84 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (27) an amino acid residue at position 85 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or threonine; and/or
- (28) an amino acid residue at position 86 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine or isoleucine; and/or
- (29) an amino acid residue at position 87 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is

- deleted or substituted with alanine, cysteine, phenylalanine, isoleucine, leucine, methionine, valine or tryptophan; and/or
- (30) an amino acid residue at position 90 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
 - (31) an amino acid residue at position 91 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
 - (32) an amino acid residue at position 99 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine or proline; and/or
 - (33) an amino acid residue at position 101 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or leucine; and/or
 - (34) an amino acid residue at position 102 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
 - (35) an amino acid residue at position 103 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, leucine or methionine; and/or
 - (36) an amino acid residue at position 105 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, leucine or tryptophan; and/or
 - (37) an amino acid residue at position 106 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
 - (38) an amino acid residue at position 108 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with lysine, arginine or tryptophan; and/or
 - (39) an amino acid residue at position 111 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
 - (40) an amino acid residue at position 117 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or
 - (41) an amino acid residue at position 120 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine or lysine; and/or

- (42) an amino acid residue at position 119 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (43) an amino acid residue at position 126 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline; and/or
- (44) an amino acid residue at position 132 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (45) an amino acid residue at position 141 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartic acid; and/or
- (46) an amino acid residue at position 146 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (47) an amino acid residue at position 149 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine or serine; and/or
- (48) an amino acid residue at position 154 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with lysine; and/or
- (49) an amino acid residue at position 159 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (50) an amino acid residue at position 160 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (51) an amino acid residue at position 162 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline, histidine or asparagine; and/or
- (52) an amino acid residue at position 175 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine, lysine, proline, glutamine, serine, threonine or tryptophan; and/or
- (53) an amino acid residue at position 176 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or

- (54) an amino acid residue at position 187 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
- (55) an amino acid residue at position 189 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (56) an amino acid residue at position 193 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, threonine or valine; and/or
- (57) an amino acid residue at position 206 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (58) an amino acid residue at position 211 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid; and/or
- (59) an amino acid residue at position 213 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline or leucine; and/or
- (60) an amino acid residue at position 214 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, threonine or valine, histidine, glutamic acid, arginine or phenylalanine; and/or
- (61) an amino acid residue at position 215 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (62) an amino acid residue at position 216 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (63) an amino acid residue at position 222 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (64) an amino acid residue at position 228 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine, alanine, proline, threonine or valine; and/or
- (65) an amino acid residue at position 232 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or

- (66) an amino acid residue at position 244 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine; and/or
- (67) an amino acid residue at position 247 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or
- (68) an amino acid residue at position 264 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartic acid; and/or
- (69) an amino acid residue at position 278 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or
- (70) an amino acid residue at position 284 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine or leucine; and/or
- (71) an amino acid residue at position 285 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (72) an amino acid residue at position 303 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine or proline; and/or
- (73) an amino acid residue at position 305 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or aspartic acid; and/or
- (74) an amino acid residue at position 306 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, arginine or serine; and/or
- (75) an amino acid residue at position 326 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or proline; and/or
- (76) an amino acid residue at position 338 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline, alanine or serine; and/or
- (77) an amino acid residue at position 341 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or

- (78) an amino acid residue at position 342 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or
- (79) an amino acid residue at position 345 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (80) an amino acid residue at position 349 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
- (81) an amino acid residue at position 352 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine or leucine; and/or
- (82) an amino acid residue at position 375 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
- (83) an amino acid residue at position 377 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine; and/or
- (84) an amino acid residue at position 384 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine; and/or
- (85) an amino acid residue at position 386 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (86) an amino acid residue at position 392 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine or alanine; and/or
- (87) an amino acid residue at position 395 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
- (88) an amino acid residue at position 399 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine or asparagine; and/or
- (89) an amino acid residue at position 402 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine or histidine; and/or

- (90) an amino acid residue at position 404 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine or tryptophan; and/or
- (91) an amino acid residue at position 406 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamine; and/or
- (92) an amino acid residue at position 414 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (93) an amino acid residue at position 440 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or
- (94) an amino acid residue at position 443 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (95) an amino acid residue at position 448 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine, phenylalanine or tryptophan; and/or
- (96) an amino acid residue at position 454 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or
- (97) an amino acid residue at position 460 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine or proline; and/or
- (98) an amino acid residue at position 461 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine, asparagine or methionine; and/or
- (99) an amino acid residue at position 462 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
- (100) an amino acid residue at position 484 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or glycine; and/or
- (101) an amino acid residue at position 488 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or asparagine; and/or

- (102) an amino acid residue at position 493 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
 - (103) an amino acid residue at position 494 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with arginine; and/or
 - (104) an amino acid residue at position 496 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or phenylalanine; and/or
 - (105) an amino acid residue at position 429 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or serine; and/or
 - (106) an amino acid residue at position 442 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or
 - (107) an amino acid residue at position 445 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid or proline; and/or
 - (108) an amino acid residue at position 501 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid, methionine, glycine or lysine; and/or
 - (109) an amino acid residue at position 502 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
 - (110) an amino acid residue at position 509 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or
 - (111) an amino acid residue at position 512 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamic acid, histidine or serine.
- 5. A nucleic acid molecule encoding the MDC variant of any one of claims 1 to 4.
 - 6. A vector comprising the nucleic acid molecule of claim 5.
 - 7. A host cell comprising the nucleic acid molecule of claim 5 or the vector of claim 6.

8. Use of the MDC variant of any one of claims 1 to 4 or the host cell of claim 7 for the conversion of 3-methylcrotonic acid into isobutene.
9. A method for producing isobutene from 3-methylcrotonic acid by incubating 3-methylcrotonic acid with the MDC variant of any one of claims 1 to 4.
10. The method of claim 9, wherein the enzymatic conversion is carried out in vitro.
11. A composition comprising a variant of an MDC of any one of claims 1 to 4, the nucleic acid molecule of claim 5, the vector of claim 6 or the host cell of claim 7.
12. A composition comprising a variant of an MDC of any one of claims 1 to 4, the nucleic acid molecule of claim 5, the vector of claim 6 or the host cell of claim 7 and 3-methylcrotonic acid.

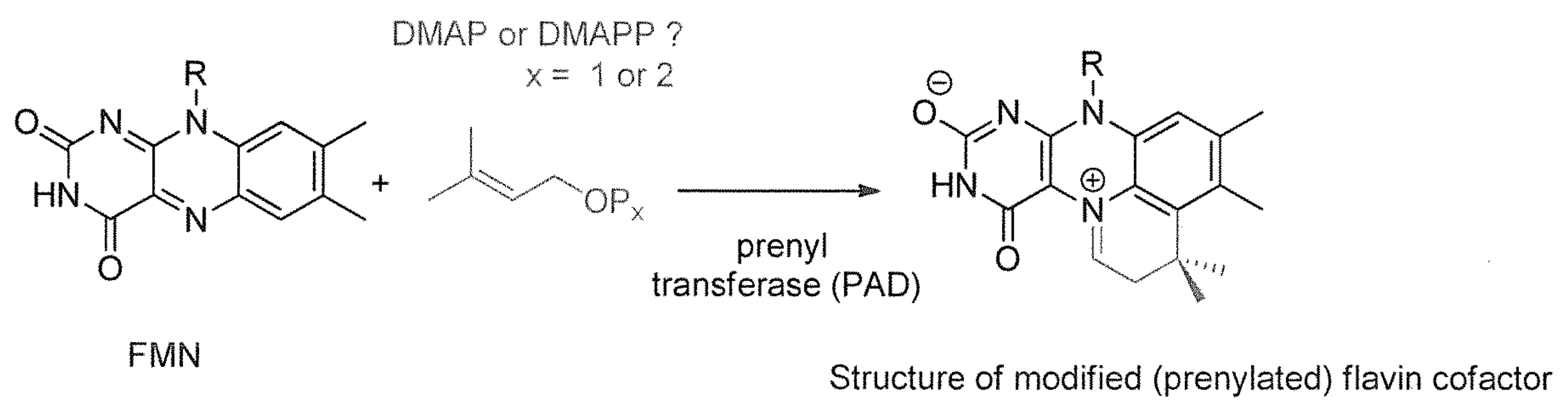


Figure 1A

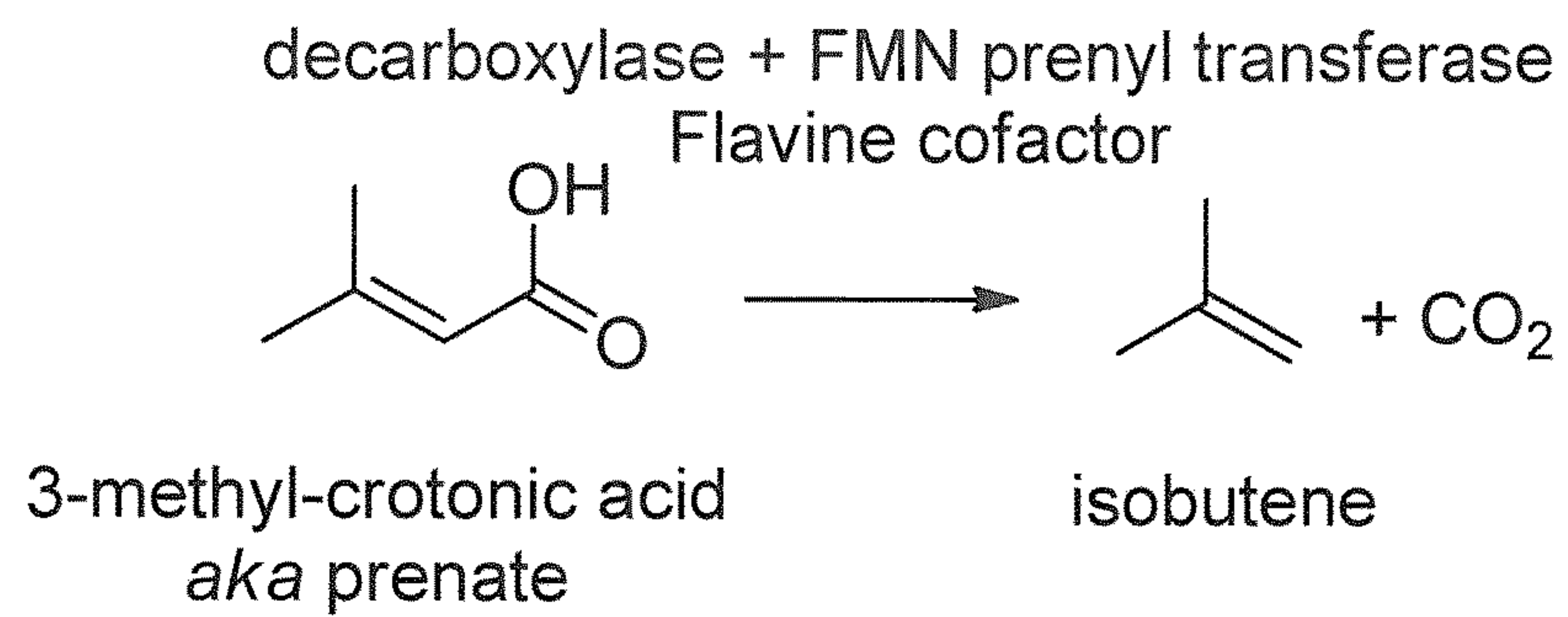


Figure 1B

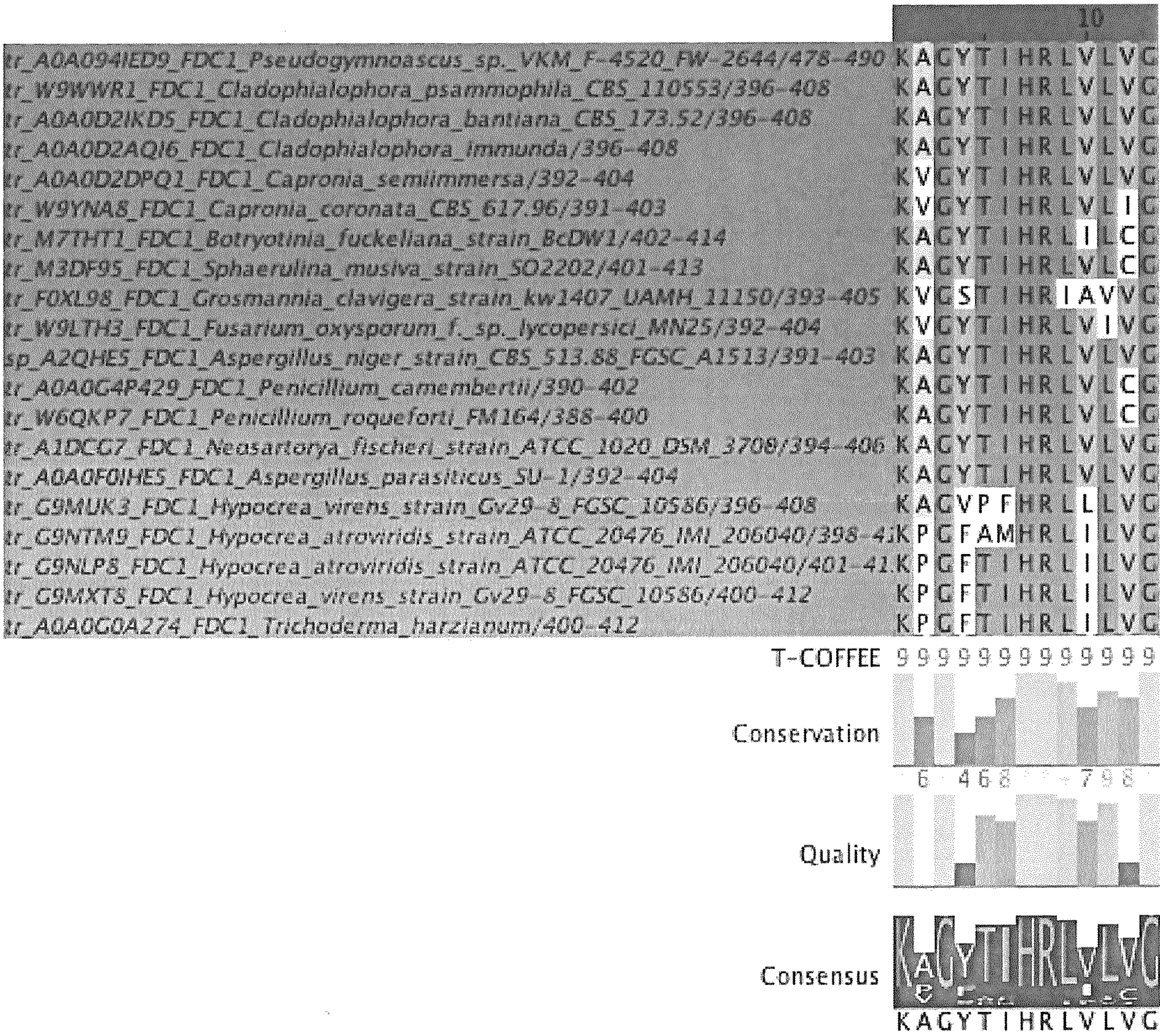


Figure 2

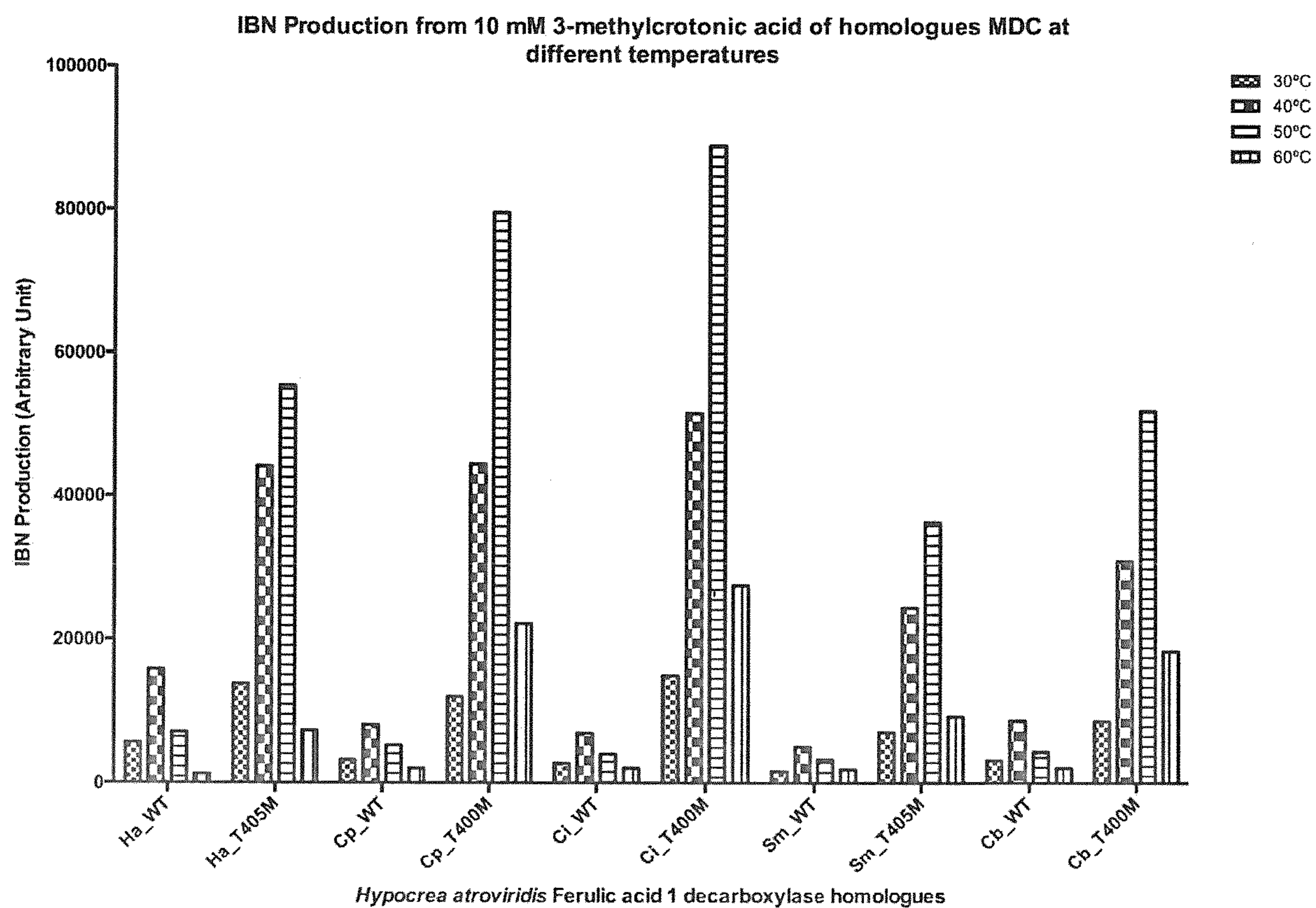


Figure 3