



(51) International Patent Classification:

C12N 9/08 (2006.01) *C12P 7/22* (2006.01)
C12N 9/02 (2006.01) *C12P 7/46* (2006.01)
C12N 9/88 (2006.01) *C12R 1/01* (2006.01)

(21) International Application Number:

PCT/EP2016/064752

(22) International Filing Date:

24 June 2016 (24.06.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

15305972.0 24 June 2015 (24.06.2015) EP

(71) Applicant: **DEINOVE** [FR/FR]; 1682 rue de la Valsière
Cap Sigma-Zac Euromédecine II, 34790 Grabels (FR).

(72) Inventors: **LEONETTI, Jean-Paul**; 9 rue Claude Monet,
34170 Castelnau Le Lez (FR). **HIVIN, Patrick**; Le Clos
des Garrigues 12 rue des Euphorbes, 34430 Saint Jean de
Védas (FR).

(74) Agent: **PIERRU, Bénédicte**; Cabinet Becker et Associes,
25, rue Louis le Grand, 75002 Paris (FR).

(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, 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, KN, KP, KR,
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: METHOD OF PRODUCING MUCONIC ACID

(57) Abstract: The present invention relates to a recombinant *Deinococcus* bacterium comprising a heterologous biosynthetic path-
way converting 3-dehydroshikimate to catechol and/or cis,cis-muconic acid and its use for producing cis,cis-muconic acid or cat-
echol, and derivatives thereof.



METHOD OF PRODUCING MUCONIC ACID

FIELD OF THE INVENTION

The present invention relates to the field of microbiology. More particularly, the present invention relates to the field of production of catechol and muconic acid from
5 renewable carbon resources using genetically modified bacteria.

BACKGROUND OF THE INVENTION

Adipic acid is the most important commercial aliphatic dicarboxylic acid in the chemical industry. It is mainly a precursor used for the production of nylon, lubricants, coating, plastics and plasticizers.

10 To date, almost all of the commercial adipic acid is still derived from petro-chemistry based precursors. Mainly, the process starts with the oxydation of benzene-derived cyclohexane resulting in a cyclohexanol-cyclohexanone mixture that is further oxidized by nitric acid (Musser, 2005). However, this process depends on finite fossil resources and has a heavy environmental impact due to a high energy input, the
15 production of large amount of the greenhouse gas N₂O and the toxicity of the chemical intermediates.

In the past few years, efforts were made to find alternative routes for adipic acid production from renewable carbon sources. Among the different strategies, a possible replacement precursor is the metabolic intermediate *cis-cis*-muconic acid (ccMA) which
20 can be efficiently converted to adipic acid via hydrogenation (Niu et al., 2002). The first synthetic route using glucose as carbon source has been established in *E. coli* and is based on the expression of three heterologous genes that encode a 3-dehydroshikimate dehydratase (AroZ) and a protocatechuate decarboxylase (AroY) from *Klebsiella pneumoniae* and a catechol 1,2-dioxygenase (CatA) from *Acinetobacter calcoaceticus*
25 (Niu et al., 2002). However, even optimized, this genetically modified bacterium does not allow a cost-competitive industrial production process. Alternatively, biotechnological production of ccMA has also been implemented in *Saccharomyces cerevisiae* by

introducing a pathway similar to that previously used in *E. coli*. However, the production titers of ccMA obtained with *S. cerevisiae* are much lower than those obtained with *E. coli* (Weber et al., 2012).

Consequently, there is still a strong need for a much improved process achieving industrially relevant productivity of muconic acid from renewable carbon sources, and in particular from lignocellulosic biomasses.

SUMMARY OF THE INVENTION

Based on their solid knowledge of *Deinococcus* metabolism and genetics, the inventors demonstrated that *Deinococcus* bacteria can be genetically modified to produce substantial amounts of catechol and muconic acid.

Accordingly, in a first aspect, the present invention relates to a recombinant *Deinococcus* bacterium expressing a heterologous polypeptide exhibiting 3-dehydroshikimate dehydratase activity and a heterologous polypeptide exhibiting protocatechuate decarboxylase activity, and optionally further expressing a heterologous polypeptide exhibiting catechol 1,2-dioxygenase activity. The present invention also relates to a recombinant *Deinococcus* bacterium comprising a heterologous nucleic acid sequence encoding a polypeptide exhibiting 3-dehydroshikimate dehydratase activity and a heterologous nucleic acid sequence encoding a polypeptide exhibiting protocatechuate decarboxylase activity, and optionally a heterologous nucleic acid sequence encoding a polypeptide exhibiting catechol 1,2-dioxygenase activity.

The polypeptide exhibiting 3-dehydroshikimate dehydratase activity may be selected, for example, from the group consisting of 3-dehydroshikimate dehydratases from *Bacillus thuringiensis*, *Podospora anserina*, *Klebsiella pneumoniae*, *Acinetobacter calcoaceticus*, *Acinetobacter* sp. ADP1, *Acinetobacter baylyi*, *Neurospora crassa*, *Aspergillus nidulans*, *Gluconobacter oxydans* and *Pseudomonas putida*, in particular *Pseudomonas putida* KT2440 and *Pseudomonas putida* H8234, preferably selected from the group consisting of *Bacillus thuringiensis*, *Podospora anserina*, *Pseudomonas putida* and *Acinetobacter* sp. ADP1, more preferably from *Bacillus thuringiensis*, *Podospora*

anserina and *Acinetobacter* sp. ADP1, and even more preferably from *Bacillus thuringiensis* and *Acinetobacter* sp. ADP1.

In particular, the polypeptide exhibiting 3-dehydroshikimate dehydratase activity may be selected from the group consisting of 3-dehydroshikimate dehydratases of SEQ ID NO: 4, 2, 6 and 8, and polypeptides exhibiting 3-dehydroshikimate dehydratase activity and having at least 60 % identity to SEQ ID NO: 4, 2, 6 or 8, preferably from the group consisting of 3-dehydroshikimate dehydratases of SEQ ID NO: 4 and 8, and polypeptides exhibiting 3-dehydroshikimate dehydratase activity and having at least 60 % identity to SEQ ID NO: 4 or 8.

The polypeptide exhibiting protocatechuate decarboxylase activity may be selected, for example, from the group consisting of 3-protocatechuate decarboxylases from *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Sedimentibacter hydroxybenzoicus*, preferably from *Klebsiella pneumoniae*.

Preferably, the polypeptide exhibiting protocatechuate decarboxylase activity is selected from the group consisting of (i) a PCA decarboxylase comprising polypeptides of SEQ ID NO: 10, 12 and 14, (ii) a PCA decarboxylase comprising polypeptides of SEQ ID NO: 17, 19 and 21, (iii) the PCA decarboxylase of SEQ ID NO: 23, and (iv) PCA decarboxylases having at least 60 % identity to SEQ ID NO: 10, 12, 14, 17, 19, 21 or 23. More preferably, the polypeptide exhibiting protocatechuate decarboxylase activity is selected from the group consisting of a PCA decarboxylase comprising polypeptides of SEQ ID NO: 10, 12 and 14, and PCA decarboxylases having at least 60 % identity to SEQ ID NO: 10, 12 or 14.

The polypeptide exhibiting catechol 1,2-dioxygenase activity may be selected, for example, from the group consisting of catechol 1,2-dioxygenases from *Acinetobacter radioresistens*, *Acinetobacter calcoaceticus*, *Candida albicans*, *Bulkholderia mallei*, *Bulkholderia xenovorans*, *Pseudomonas putida*, *Stenotrophomonas maltophilia* KB2, *Cupriavidus metallidurans* CH34, *Burkholderia* sp. TH2, *Rhodococcus opacus*, *Rhodococcus erythropolis* and *Acinetobacter* sp. ADP1. Preferably, the polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenases from *Acinetobacter radioresistens*, *Acinetobacter calcoaceticus*, *Candida albicans*, *Bulkholderia mallei*, *Bulkholderia xenovorans* and

Pseudomonas putida, more preferably from *Acinetobacter radioresistens*, *Acinetobacter calcoaceticus*, *Candida albicans*, *Bulkholderia mallei* and *Bulkholderia xenovorans*.

In particular, the polypeptide exhibiting catechol 1,2-dioxygenase activity may be selected from the group consisting of catechol 1,2-dioxygenases of SEQ ID NO: 25, 27, 5 29, 31, 33, 34, 35, 36, 37, 38, 39, 40 and 41 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 25, 27, 29, 31, 33, 34, 35, 36, 37, 38, 39, 40 or 41. Preferably, the polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenases of SEQ ID NO: 25, 29 and 31 and polypeptides exhibiting catechol 1,2-dioxygenase 10 activity and having at least 60 % identity to SEQ ID NO: 25, 29 or 31. More preferably, the polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 25 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 25.

Alternatively, the polypeptide exhibiting catechol 1,2-dioxygenase activity may 15 be selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 41 and a variant thereof comprising at least one substitution at position corresponding to residue G72, L73 or P76, preferably at least one substitution or combination of substitutions selected from G72A, L73F, P76A, G72A+L73F, G72A+P76A, P76A+L73F, G72A+L72F+P76A, more preferably selected from G72A, P76A, L73F and L73F+P76A.

20 The endogenous biosynthetic pathway of the bacterium converting 3-dehydroshikimate to chorismate may be blocked or reduced. In particular, the endogenous gene encoding shikimate dehydrogenase (AroE) may be inactivated. Alternatively, the shikimate dehydrogenase activity of the bacterium may be reduced.

The endogenous biosynthetic pathway converting protocatechuate to oxoadipate 25 may be blocked or reduced. In particular, this pathway may be blocked by inactivation one or several endogenous genes selected from genes encoding protocatechuate 3,4-dioxygenase, 3-carboxy-cis,cis-muconate cycloisomerase and 3-oxoadipate enol-lactonase.

One or several of the enzymes involved in the conversion of phosphoenolpyruvate 30 and erythrose 4-phosphate to 3-dihydroshikimate, preferably selected from AroF, AroG,

AroH, AroB and AroD, may be overexpressed and/or may be feedback inhibition resistant enzymes.

In particular, the recombinant bacterium may express a feedback inhibition resistant DAHP synthase, preferably a variant of the *Deinococcus* DAHP synthase set forth in SEQ ID NO: 46 comprising at least one substitution at position corresponding to residue N13, P156 or S186 of SEQ ID NO: 46, preferably selected from N13K, P156L, S186F, N13K+ P156L, N13K+S186F, P156L +S186F and N13K+ P156L +S186F.

The recombinant bacterium of the invention may further express a heterologous polypeptide exhibiting catechol-O-methyltransferase activity. Preferably, said polypeptide is selected from the group consisting of COMT from *Mycobacterium vanbaalenii* (SEQ ID NO: 54) and any polypeptide exhibiting COMT activity and having at least 60 % identity to SEQ ID NO: 54.

In a second aspect, the present invention also relates to a method of producing *cis-cis* muconic acid comprising culturing a recombinant *Deinococcus* bacterium of the invention under conditions suitable to produce *cis-cis* muconic acid, and optionally recovering said *cis-cis* muconic acid.

In another aspect, the present invention also relates to a method of producing catechol comprising culturing a recombinant *Deinococcus* bacterium of the invention under conditions suitable to produce catechol, and optionally recovering said catechol.

In a further aspect, the present invention also relates to a method of producing *cis-cis* muconic acid comprising (i) producing catechol according to the method of producing catechol of the invention, (ii) enzymatically converting catechol to *cis-cis* muconic acid, and optionally (iii) recovering said *cis-cis* muconic acid.

In the methods of the invention, the culture of the recombinant *Deinococcus* bacterium under conditions suitable to produce *cis-cis* muconic acid may be performed at a temperature comprised between 37 and 55°C, preferably at about 48°C.

In the methods of the invention, the culture of the recombinant *Deinococcus* bacterium under conditions suitable to produce catechol may be performed at a temperature comprised between 37 and 55°C, preferably at about 37°C.

In another aspect, the present invention relates to a method of producing adipic acid comprising producing *cis-cis* muconic acid according to the method of the invention and reducing said *cis-cis* muconic acid to produce adipic acid, and optionally recovering said adipic acid.

5 In another aspect, the present invention relates to a method of producing *cis-trans* and/or *trans-trans* muconic acid comprising producing *cis-cis* muconic acid according to the method of the invention and isomerizing said *cis-cis* muconic acid to produce *cis-trans* and/or *trans-trans* muconic acid, and optionally recovering said *cis-trans* and/or *trans-trans* muconic acid.

10 In a further aspect, the present invention also relates to a method of producing gaiacol comprising (i) culturing a recombinant *Deinococcus* bacterium of the invention expressing a heterologous polypeptide exhibiting catechol-O-methyltransferase activity under conditions suitable to produce gaiacol, and optionally (ii) recovering said gaiacol.

The present invention further relates to a method of producing vanillin comprising
15 (i) culturing a recombinant *Deinococcus* bacterium of the invention expressing a heterologous polypeptide exhibiting catechol-O-methyltransferase activity under conditions suitable to produce gaiacol, (ii) converting gaiacol to vanillin and, optionally (iii) recovering said vanillin. The method may further comprise recovering gaiacol produced in step (i) before conversion.

20 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Expression cassette for the production of catechol comprising *AroZ* of *Bacillus thuringiensis*, *aroY* of *Klebsiella pneumonia* and a gene encoding bleomycin resistance. All these genes were placed under the control of strong constitutive promoters. The cassette further comprised two flanking sequences of 1500bp that are homologous to
25 the sequence upstream and downstream the chromosomal target *aroE* gene.

Figure 2: Titters of DHS, PCA and catechol of recombinant *D. geothermalis* comprising the cassette of Figure 1. Wild-type strain does not produce PCA or catechol (data not shown).

Figure 3: Expression cassette for the production of catechol comprising *quiC* of *Acinetobacter* sp. ADP1, *aroY* of *Klebsiella pneumonia* and a gene encoding bleomycin resistance. All these genes were placed under the control of strong constitutive promoters. The cassette further comprised two flanking sequences of 1500bp that are homologous to the sequence upstream and downstream the chromosomal target *aroE* gene.

Figure 4: HPLC analysis of the culture sample of the recombinant *Deinococcus geothermalis* comprising the expression cassette of Figure 1 and an expression cassette comprising *catA* gene from *Acinetobacter calcoaceticus*. The wild-type strain does not produce muconic acid (data not shown).

Figure 5: Titers of DHS, catechol and muconic acid of recombinant *D. geothermalis* strains comprising the expression cassette of Figure 1 (in grey) or Figure 3 (in white) and an expression cassette comprising *catA* gene from *Acinetobacter calcoaceticus*. Wild-type strains do not produce catechol or muconic acid (data not shown).

DETAILED DESCRIPTION OF THE INVENTION

Deinococcus bacteria are non-pathogen bacteria that were firstly isolated in 1956 by Anderson and collaborators. These extremophile organisms have been proposed for use in industrial processes or reactions using biomass (see e.g., WO2009/063079; WO2010/094665 or WO2010/081899).

Based on their solid knowledge of *Deinococcus* metabolism and genetics, the inventors found that *Deinococcus* bacteria can be genetically modified to produce substantial amounts of muconic acid and exhibit specific properties that are particularly useful for industrial production of this compound. Indeed, *Deinococcus* bacteria are viable at a pH comprised between 4 and 9, they are thus resistant to the decrease of pH values induced by the production of organic acid. *Deinococcus* bacteria are also able to grow on and/or transform a very large variety of organic substrates, including cellulosic biomass (see e.g. the international patent application WO 2010/130812), thus allowing industrial production from biorenewables. Furthermore, *Deinococcus* bacteria may exhibit a natural balance between the oxidative pentose phosphate pathway and the

glycolysis pathway which promotes the adequate supplying of molecules that are precursors of 3-dehydroshikimate such as phosphoenolpyruvate (PEP) and D-erythrose 4-phosphate (E4P).

Definitions

- 5 In the context of the invention, the term “*Deinococcus*” includes wild type or natural variant strains of *Deinococcus*, e.g., strains obtained through accelerated evolution, mutagenesis, by DNA-shuffling technologies, or recombinant strains obtained by insertion of eukaryotic, prokaryotic and/or synthetic nucleic acid(s). *Deinococcus* bacteria can designate any bacterium of the genus *Deinococcus*, such as without
- 10 limitation, *D. aerius*, *D. aerolatus*, *D. aerophilus*, *D. aetherius*, *D. alpinitundrae*, *D. altitudinis*, *D. antarcticus*, *D. apachensis*, *D. aquaticus*, *D. aquaticus*, *D. aquatilis*, *D. aquiradiocola*, *D. caeni*, *D. cellulolysiticus*, *D. citri*, *D. claudionis*, *D. daejeonensis*, *D. depolymerans*, *D. desertii*, *D. enclensis*, *D. ficus*, *D. frigens*, *D. geothermalis*, *D. gobiensis*, *D. grandis*, *D. guangriensis*, *D. guilhemensis*, *D. hohokamensis*, *D. hopiensis*,
- 15 *D. humi*, *D. indicus*, *D. maricopenis*, *D. marmoris*, *D. misasensis*, *D. murrayi*, *D. navajonensis*, *D. papagonensis*, *D. peraridilitoris*, *D. phoenicis*, *D. pimensis*, *D. piscis*, *D. proteolyticus*, *D. puniceus*, *D. radiodurans*, *D. radiomollis*, *D. radiophilus*, *D. radiopugnans*, *D. radioresistens*, *D. radiotolerans*, *D. reticulitermitis*, *D. roseus*, *D. sahariensis*, *D. saxicola*, *D. soli*, *D. sonorensis*, *D. swuensis*, *D. wulumuqiensis*, *D.*
- 20 *xinjiangensis* and *D. yavapaiensis* bacterium, or any combinations thereof. Preferably, the term “*Deinococcus*” refers to *D. geothermalis*, *D. cellulolysiticus*, *D. deserti*, *D. murrayi*, *D. maricopenis* or *D. radiodurans*. More preferably, the term “*Deinococcus*” refers to *D. geothermalis*. Examples of mesophilic *Deinococcus* bacteria include, but are not limited to, *D. radiodurans*, *D. grandis*, *D. cellulolysiticus*, *D. depolymerans*, *D.*
- 25 *aquaticus*, *D. deserti*, *D. wulumuqiensis*, *D. proteolyticus*, *D. gobiensis*, *D. misasensis*, *D. frigens*, *D. marmoris*, *D. ficus*, *D. apachensis*, *D. aquatilis*, *D. pimensis*, *D. peraridilitoris*, *D. puniceus*, *D. phoenicis*, *D. swuensis* and *D. actinosclerus*. Examples of thermophilic *Deinococcus* bacteria include, but are not limited to, *D. geothermalis*, *D. maricopenis* and *D. murrayi*.
- 30 The term “recombinant bacterium” or “genetically modified bacterium” designates a bacterium that is not found in nature and which contains a modified genome

as a result of either a deletion, insertion or modification of genetic elements. A "recombinant nucleic acid" therefore designates a nucleic acid which has been engineered and is not found as such in wild type bacteria.

The term "gene" designates any nucleic acid encoding a protein. The term gene
5 encompasses DNA, such as cDNA or gDNA, as well as RNA. The gene may be first prepared by e.g., recombinant, enzymatic and/or chemical techniques, and subsequently replicated in a host cell or an *in vitro* system. The gene typically comprises an open reading frame encoding a desired protein. The gene may contain additional sequences such as a transcription terminator or a signal peptide.

10 The term "expression cassette" denotes a nucleic acid construct comprising a coding region, i.e. a gene, and a regulatory region, i.e. comprising one or more control sequences, operably linked. Preferably, the control sequences are suitable for *Deinococcus* host cells.

As used herein, the term "expression vector" means a DNA or RNA molecule that
15 comprises an expression cassette. Preferably, the expression vector is a linear or circular double stranded DNA molecule.

The term "operably linked" means a configuration in which a control sequence is placed at an appropriate position relative to a coding sequence, in such a way that the control sequence directs expression of the coding sequence.

20 The term "control sequences" means nucleic acid sequences necessary for expression of a gene. Control sequences may be native, homologous or heterologous. Well-known control sequences and currently used by the person skilled in the art will be preferred. Such control sequences include, but are not limited to, a leader, polyadenylation sequence, propeptide sequence, promoter, signal peptide sequence, and
25 transcription terminator. Preferably, the control sequences include a promoter and a transcription terminator.

As used herein, the term "native" or "endogenous" refers to a genetic element or a protein from the non modified *Deinococcus* bacterium or from a *Deinococcus* bacterium of the same species. The term "homologous" refers to a genetic element or a protein from
30 a *Deinococcus* bacterium of another species than the recombinant *Deinococcus*

bacterium. The term “heterologous” refers to a genetic element or a protein from a non *Deinococcus* origin such as other bacteria, microorganisms, plants, viruses, ect...

As used herein, the term “sequence identity” or “identity” refers to the number (%) of matches (identical amino acid residues) in positions from an alignment of two polypeptide sequences. The sequence identity is determined by comparing the sequences when aligned so as to maximize overlap and identity while minimizing sequence gaps. In particular, sequence identity may be determined using any of a number of mathematical global or local alignment algorithms, depending on the length of the two sequences. Sequences of similar lengths are preferably aligned using a global alignment algorithms (e.g. Needleman and Wunsch algorithm; Needleman and Wunsch, 1970) which aligns the sequences optimally over the entire length, while sequences of substantially different lengths are preferably aligned using a local alignment algorithm (e.g. Smith and Waterman algorithm (Smith and Waterman, 1981) or Altschul algorithm (Altschul et al., 1997; Altschul et al., 2005)). Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software available on internet web sites such as <http://blast.ncbi.nlm.nih.gov/> or <http://www.ebi.ac.uk/Tools/emboss/>). Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, % amino acid sequence identity values refers to values generated using the pair wise sequence alignment program EMBOSS Needle that creates an optimal global alignment of two sequences using the Needleman-Wunsch algorithm, wherein all search parameters are set to default values, i.e. Scoring matrix = BLOSUM62, Gap open = 10, Gap extend = 0.5, End gap penalty = false, End gap open = 10 and End gap extend = 0.5

The term “AroZ” or “DHS dehydratase” refers to the enzyme 3-dehydroshikimate dehydratase (EC 4.2.1.118) encoded by *aroZ* gene, that converts 3-dehydroshikimate (DHS) to protocatechuate (PCA).

The term “AroY” or “PCA decarboxylase” refers to the enzyme protocatechuate decarboxylase (also named 3,4-dihydroxybenzoate decarboxylase or protocatechuate carboxylase, EC 4.1.1.63) encoded by *aroY* gene, that converts protocatechuate (also

named 3,4-dihydroxybenzoic acid) to catechol (also named Benzene-1,2-diol, Pyrocatechol, 2-hydroxyphenol, or 1,2-dihydroxybenzene).

The term “CatA” refers to the enzyme catechol 1,2-dioxygenase (also named catechase or pyrocatechase, EC 1.13.11.1) encoded by *catA* gene, that converts catechol
5 to *cis,cis*-muconic acid (ccMA).

The term “AroE” refers to the enzyme shikimate dehydrogenase (also named 5-dehydroshikimate reductase, EC 1.1.1.25) encoded by *aroE* gene, that converts DHS to shikimate (SHK).

The terms “AroK” refers to the enzyme shikimate kinase (also named
10 ATP:shikimate 3-phosphotransferase, EC 2.7.1.71) encoded by *aroK* gene. This enzyme converts SHK to 3-phosphoshikimate (S3P).

The term “AroA” refers to the enzyme 3-phosphoshikimate 1-carboxyvinyltransferase (also named 5-enolpyruvylshikimate-3-phosphate synthase, 3-enol-pyruvoylshikimate-5-phosphate synthase or EPSP synthase, EC 2.5.1.19) encoded
15 by *aroA* gene, that converts S3P to 5-enolpyruvylshikimate-3-phosphate (EPSP).

The term “AroC” refers to the enzyme chorismate synthase (also named 5-enolpyruvylshikimate-3-phosphate phospholyase, EC 4.2.3.5) encoded by *aroC* gene, that converts EPSP to chorismate (CHA).

The terms “AroG”, “AroF” and AroH” refer to DAHP synthases (also named 3-
20 deoxy-7-phosphoheptulonate synthase or Phospho-2-dehydro-3-deoxyheptonate aldolase, EC 2.5.1.54) encoded by *aroG*, *aroF* and *aroH* genes, respectively, that convert phosphoenolpyruvate (PEP) and D-erythrose 4-phosphate (E4P) to 3-deoxy-D-arabino-hept-2-ulosonate 7-phosphate (DAHP).

The term “AroB” refers to the enzyme 3-dehydroquinate synthase (EC 4.2.3.4)
25 encoded by *aroB* gene, that converts DAHP to 3-dehydroquinate (DHQ).

The term “AroD” or “AroQ” refers to the enzyme 3-dehydroquinate dehydratase (EC 4.2.1.10) encoded by *aroD* gene, that converts DHQ to DHS.

The term “Rpe” refers to ribulose-phosphate 3-epimerase (EC 5.1.3.1) encoded by the *rpe* gene, that converts D-ribulose 5-phosphate to D-xylulose 5-phosphate.

The term “Rpi” refers to ribose-5-phosphate isomerase (EC 5.3.1.6) encoded by the *rpi* gene, that converts D-ribose 5-phosphate to D-ribulose 5-phosphate.

5 The term “TalB” refers to transaldolase (EC 2.2.1.2) encoded by the *talB* gene, that converts sedoheptulose 7-phosphate and D-glyceraldehyde 3-phosphate to D-erythrose 4-phosphate and D-fructose 6-phosphate.

The term “TktA” refers to transketolase (EC 2.2.1.1) encoded by the *tktA* gene, that converts sedoheptulose 7-phosphate and D-glyceraldehyde 3-phosphate to D-ribose
10 5-phosphate and D-xylulose 5-phosphate.

The term “PpsA” refers to a Phosphoenolpyruvate (PEP) synthase (EC 2.7.9.2) encoded by the *ppsA* gene, that converts pyruvate to PEP.

The term “COMT” refers to an enzyme exhibiting catechol-O-methyltransferase activity, i.e. catalyzing the reaction converting catechol to gaiacol (EC 2.1.1.6).

15 According to the organism, the nomenclature of the above identified enzymes and encoding genes may vary. However, for the sake of clarity, in the present specification, these terms are used independently from the origin of the enzymes or genes.

Heterologous catechol and muconic acid biosynthetic pathway

In a first aspect, the present invention relates to a recombinant *Deinococcus*
20 bacterium comprising a heterologous muconic acid biosynthetic pathway. The present invention also relates to a recombinant *Deinococcus* bacterium comprising a heterologous catechol biosynthetic pathway.

As used herein, the term “biosynthetic pathway” refers to a biochemical pathway comprising one or several enzymes and converting a substrate to a product through one
25 or several biochemical reactions. In particular, the term “muconic acid biosynthetic pathway” refers to a biochemical pathway allowing the production of muconic acid, in particular *cis-cis*-muconic acid. In preferred embodiments, this term refers to a biochemical pathway converting 3-dehydroshikimate (DHS) to *cis-cis*-muconic acid

(ccMA), and in particular a biochemical pathway converting DHS to protocatechuate (PCA), PCA to catechol and catechol to ccMA. All or part of the biochemical pathway converting DHS to ccMA may be heterologous. Thus, the recombinant *Deinococcus* bacterium of the invention may comprise a heterologous nucleic acid sequence encoding
5 a polypeptide exhibiting 3-dehydroshikimate dehydratase activity (*aroZ* gene), a heterologous nucleic acid sequence encoding a polypeptide exhibiting protocatechuate decarboxylase activity (*aroY* gene), and/or a heterologous nucleic acid sequence encoding a polypeptide exhibiting catechol 1,2-dioxygenase activity (*catA* gene).

The term “catechol biosynthetic pathway” refers to a biochemical pathway
10 allowing the production of catechol. In preferred embodiments, this term refers to a biochemical pathway converting 3-dehydroshikimate (DHS) to protocatechuate (PCA) and PCA to catechol. All or part of the biochemical pathway converting DHS to catechol may be heterologous. Thus, the recombinant *Deinococcus* bacterium of the invention comprising a heterologous catechol biosynthetic pathway may comprise a heterologous
15 nucleic acid sequence encoding a polypeptide exhibiting 3-dehydroshikimate dehydratase activity (*aroZ* gene) and/or a heterologous nucleic acid sequence encoding a polypeptide exhibiting protocatechuate decarboxylase activity (*aroY* gene). In a preferred embodiment, the recombinant *Deinococcus* bacterium of the invention comprising a heterologous catechol biosynthetic pathway does not exhibit any catechol 1,2-
20 dioxygenase activity.

The polypeptide exhibiting DHS dehydratase activity (AroZ) may be any known DHS dehydratase, in particular selected from known fungal or bacterial DHS dehydratases. Preferably, the polypeptide exhibiting DHS dehydratase activity is selected from the group consisting of DHS dehydratases from *Bacillus thuringiensis* (AsbF, Fox
25 et al., 2008; SEQ ID NO: 4), *Podospira anserina* (also known as *Podospira pauciseta*; Hansen et al. 2009; SEQ ID NO: 2), *Pseudomonas putida*, in particular *Pseudomonas putida* KT2440 (Jimenez et al. 2002) and *Pseudomonas putida* H8234 (Molina et al., 2013; SEQ ID NO: 6), *Klebsiella pneumonia* (Draths et al., 1995; Niu et al., 2002), *Acinetobacter calcoaceticus* (quiC, Elsemore et al., 1995), *Acinetobacter sp. ADPI* and
30 *Acinetobacter baylyi* (quiC, SEQ ID NO: 8), *Neurospora crassa* (Qa-4, Rutledge et al., 1984), *Aspergillus nidulans* (QutC, Lamb et al., 1992) and *Gluconobacter oxydans* (DSD, Shinagawa et al., 2010). More preferably, the polypeptide exhibiting DHS dehydratase

activity is selected from the group consisting of DHS dehydratases from *Bacillus thuringiensis*, *Podospira anserina*, *Pseudomonas putida* H8234 and KT2440, *Klebsiella pneumonia*, *Acinetobacter baylyi*, *Acinetobacter calcoaceticus*, *Acinetobacter* sp. ADP1 and *Neurospora crassa*. Even more preferably, the polypeptide exhibiting DHS dehydratase activity is selected from the group consisting of DHS dehydratases from *Bacillus thuringiensis* (SEQ ID NO: 4), *Podospira anserina* (SEQ ID NO: 2), *Pseudomonas putida* H8234 (SEQ ID NO: 6) and *Acinetobacter* sp. ADP1 (SEQ ID NO: 8). In a particular embodiment, the polypeptide exhibiting DHS dehydratase activity is selected from the group consisting of DHS dehydratases from *Bacillus thuringiensis* (SEQ ID NO: 4) and *Acinetobacter* sp. ADP1 (SEQ ID NO: 8). In particular, the AroZ enzyme may be any polypeptide exhibiting DHS dehydratase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, and more preferably at least 90 or 95%, identity to any DHS dehydratase listed above.

In a particular embodiment, the AroZ enzyme is selected from the group consisting of DHS dehydratases of SEQ ID NO: 2, 4, 6 and 8 and polypeptides exhibiting DHS dehydratase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, and more preferably at least 90 or 95% identity to SEQ ID NO: 2, 4, 6 or 8. Preferably, the AroZ enzyme is selected from the group consisting of DHS dehydratases of SEQ ID NO: 2, 4 and 8 and polypeptides exhibiting DHS dehydratase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, and more preferably at least 90 or 95% identity to SEQ ID NO: 2, 4 or 8. More preferably, the AroZ enzyme is selected from the group consisting of DHS dehydratases of SEQ ID NO: 4 and 8 and polypeptides exhibiting DHS dehydratase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, and more preferably at least 90 or 95% identity to SEQ ID NO: 4 or 8. Even more preferably, the AroZ enzyme is selected from the group consisting of DHS dehydratases of SEQ ID NO: 4 and 8.

The polypeptide exhibiting PCA decarboxylase activity (AroY) may be any known PCA decarboxylase, in particular selected from known fungal or bacterial PCA decarboxylases. Preferably, the polypeptide exhibiting PCA decarboxylase activity is selected from the group consisting of PCA decarboxylases from *Klebsiella pneumonia* (Niu et al., 2002; Weber et al. 2012; three subunits: SEQ ID NO: 10, 12 and 14), *Enterobacter cloacae* (Yoshida et al., 2010; SEQ ID NO: 23), *Sedimentibacter*

hydroxybenzoicus (He et al., 1996; Weber et al., 2012; three subunits: SEQ ID NO: 17, 19 and 21) and *Aerobacter aerogenes* (Grant and Patel, 1969; Curran et al., 2013). More preferably, the polypeptide exhibiting PCA decarboxylase activity is selected from the group consisting of PCA decarboxylases from *Klebsiella pneumonia* (SEQ ID NO: 10, 12 and 14), *Enterobacter cloacae* (SEQ ID NO: 23) and *Sedimentibacter hydroxybenzoicus* (SEQ ID NO: 17, 19 and 21). Even more preferably, the polypeptide exhibiting PCA decarboxylase activity is from *Klebsiella pneumonia*. In particular, the AroY enzyme may be any polypeptide exhibiting PCA decarboxylase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, and more preferably at least 90 or 95%, identity to any PCA decarboxylase listed above.

In a particular embodiment, the AroY enzyme is selected from the group consisting of (i) a PCA decarboxylase comprising polypeptides of SEQ ID NO: 10, 12 and 14, (ii) a PCA decarboxylase comprising polypeptides of SEQ ID NO: 17, 19 and 21, (iii) the PCA decarboxylase of SEQ ID NO: 23, and (iv) PCA decarboxylases having at least 60 %, preferably at least 65, 70, 75, 80 or 85, more preferably at least 90 or 95% identity to SEQ ID NO: 10, 12, 14, 17, 19, 21 or 23. Preferably, the AroY enzyme is selected from the group consisting of a PCA decarboxylase comprising polypeptides of SEQ ID NO: 10, 12 and 14 and PCA decarboxylases having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, more preferably at least 90 or 95% identity to SEQ ID NO: 10, 12 or 14. More preferably, the AroY enzyme is a PCA decarboxylase comprising three subunits of SEQ ID NO: 10, 12 and 14.

In embodiments wherein the PCA decarboxylase comprises several (e.g. three) subunits, all subunits may be encoded by the same heterologous nucleic acid, each subunit may be encoded by distinct heterologous nucleic acid, or several (e.g. two) subunits may be encoded by a heterologous nucleic acid while the other(s) is(are) encoded by another heterologous nucleic acid.

The polypeptide exhibiting catechol 1,2-dioxygenase activity (CatA) may be any known catechol 1,2-dioxygenase, in particular selected from known fungal or bacterial catechol 1,2-dioxygenases. Preferably, the polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenases from *Acinetobacter radioresistens* (Uniprot accession numbers: Q9F103 (isoB also called

catA_B) and Q93SY8 (isoA also called catA_A; SEQ ID NO: 27); Capioso et al., 2002; Weber et al., 2012), *Acinetobacter calcoaceticus* (Uniprot accession numbers: A0A0A8XEH7 and F0KF43 ; Neidle and Ornston, 1986; SEQ ID NO: 25), *Candida albicans* (Uniprot accession numbers: A0A0A6MK79 and P86029; Tsai and Li, 2007; 5 SEQ ID NO: 29), *Bulkholderia mallei* (Uniprot accession number: Q62E52 ; SEQ ID NO: 33), *Bulkholderia xenovorans* (Weber et al., 2012; SEQ ID NO: 31), *Pseudomonas putida* (Cao et al., 2008; SEQ ID NO: 36; Genbank accession number: ABS86779.1), *Stenotrophomonas maltophilia* KB2 (SEQ ID NO: 34; Genbank accession number: ABS86780.1), *Cupriavidus metallidurans* CH34 previously known as *Ralstonia eutropha* 10 and *Alcaligenes eutrophus* (SEQ ID NO: 35 and 40; Genbank accession number: YP_587012.1 and ABF08660), *Burkholderia* sp. TH2 (SEQ ID NO: 37; Genbank accession number: BAC16779.1), *Rhodococcus opacus* (SEQ ID NO: 38; Genbank accession number: CAA67941.1), *Rhodococcus erythropolis* (SEQ ID NO: 39; Genbank accession number: BAA11859.1) and *Acinetobacter* sp. ADP1 (Uniprot accession 15 number: P07773, SEQ ID NO:41). More preferably, the polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenases from *Acinetobacter radioresistens* (Uniprot accession numbers: Q9F103 (isoB also called catA_B) and Q93SY8 (isoA also called catA_A; SEQ ID NO: 27); Capioso et al., 2002; Weber et al., 2012), *Acinetobacter calcoaceticus* (Uniprot accession 20 numbers: A0A0A8XEH7 and F0KF43 ; Neidle and Ornston, 1986; SEQ ID NO: 25), *Candida albicans* (Uniprot accession numbers: A0A0A6MK79 and P86029; Tsai and Li, 2007; SEQ ID NO: 29), *Bulkholderia mallei* (Uniprot accession number: Q62E52 ; SEQ ID NO: 33), *Bulkholderia xenovorans* (Weber et al., 2012; SEQ ID NO: 31) and *Pseudomonas putida* (Cao et al., 2008). More preferably, the polypeptide exhibiting 25 catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenases from *Acinetobacter radioresistens* (SEQ ID NO: 27), *Acinetobacter calcoaceticus* (SEQ ID NO: 25), *Candida albicans* (SEQ ID NO: 29), *Bulkholderia mallei* (SEQ ID NO: 33) and *Bulkholderia xenovorans* (SEQ ID NO: 31). Even more preferably, the polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group 30 consisting of catechol 1,2-dioxygenases from *Acinetobacter calcoaceticus* (SEQ ID NO: 25), *Candida albicans* (SEQ ID NO: 29) and *Bulkholderia xenovorans* (SEQ ID NO: 31). In a particular embodiment, the polypeptide exhibiting catechol 1,2-dioxygenase activity is from *Acinetobacter calcoaceticus*. In particular, the CatA enzyme may be any

polypeptide exhibiting catechol 1,2-dioxygenase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, more preferably at least 90 or 95%, identity to any catechol 1,2-dioxygenase listed above. Preferably, the CatA enzyme is selected from the group consisting of catechol 1,2-dioxygenases of SEQ ID NO: 25, 27, 29, 31, 33, 34, 5 35, 36, 37, 38, 39, 40 and 41 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, more preferably at least 90 or 95% identity to SEQ ID NO: 25, 27, 29, 31, 33, 34, 35, 36, 37, 38, 39, 40 or 41. More preferably, the CatA enzyme is selected from the group consisting of catechol 1,2-dioxygenases of SEQ ID NO: 25, 27, 29, 31, 33 and 41, preferably of SEQ 10 ID NO: 25, 27, 29, 31 and 33, and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, more preferably at least 90 or 95% identity to SEQ ID NO: 25, 27, 29, 31, 33 or 41, preferably to SEQ ID NO: 25, 27, 29, 31 or 33. Even more preferably, the CatA enzyme is selected from the group consisting of catechol 1,2-dioxygenases of SEQ ID NO: 25, 29 and 31, 15 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, more preferably at least 90 or 95% identity to SEQ ID NO: 25, 29 or 31. In a particular embodiment, the CatA enzyme is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 25 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 %, preferably at least 20 65, 70, 75, 80 or 85%, more preferably at least 90 or 95% identity to SEQ ID NO: 25. In a preferred embodiment, the CatA enzyme is a catechol 1,2-dioxygenase of SEQ ID NO: 25.

The CatA enzyme may be a variant of any catechol 1,2-dioxygenase listed above, said variant exhibiting improved properties such as improved activity. In an embodiment, 25 the CatA enzyme is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 41 and a variant of the enzyme set forth in SEQ ID NO: 41 comprising at least one substitution at position corresponding to residue G72, L73 or P76 of SEQ ID NO: 41 (Han et al., 2015). As used herein, the term “substitution” refers to the replacement of an amino acid residue by another selected from the naturally-occurring 30 standard 20 amino acid residues (G, P, A, V, L, I, M, C, F, Y, W, H, K, R, Q, N, E, D, S and T). The sign “+” indicates a combination of substitutions. In the present document, the following terminology is used to designate a substitution: G72A denotes that amino

acid residue at position 72 of SEQ ID No. 41 (glycine, G) is changed to an alanine (A). In a particular embodiment, the CatA enzyme is a variant of the enzyme set forth in SEQ ID NO: 41 and comprises at least one substitution at position corresponding to residue G72, L73 or P76 of SEQ ID NO: 41, preferably at least one substitution or combination of substitutions selected from G72A, L73F, P76A, G72A+L73F, G72A+P76A, P76A+L73F, G72A+L73F+P76A, more preferably selected from G72A (SEQ ID NO: 42), P76A (SEQ ID NO: 43), L73F (SEQ ID NO: 44) and L73F+P76A (SEQ ID NO: 45).

In an embodiment, the recombinant *Deinococcus* bacterium comprises a heterologous *aroZ* gene, a heterologous *aroY* gene or a heterologous *catA* gene. In another embodiment, the recombinant *Deinococcus* bacterium comprises (i) a heterologous *aroZ* gene and a heterologous *aroY* gene, (ii) a heterologous *aroZ* gene and a heterologous *catA* gene, or (iii) a heterologous *aroY* gene and a heterologous *catA* gene. In a preferred embodiment, the recombinant *Deinococcus* bacterium comprises a heterologous *aroZ* gene, a heterologous *aroY* gene and a heterologous *catA* gene.

In a particular embodiment, the recombinant *Deinococcus* bacterium comprises the *aroZ* gene and the *aroY* gene from *Klebsiella pneumoniae* and the *catA* gene from *Acinetobacter calcoaceticus* (Niu et al., 2012). In another particular embodiment, the recombinant *Deinococcus* bacterium comprises the *aroZ* gene from *Bacillus thuringiensis*, the *aroY* gene from *Klebsiella pneumoniae* and the *catA* gene from *Acinetobacter radioresistens* (Weber et al., 2012). In another particular embodiment, the recombinant *Deinococcus* bacterium comprises the *aroZ* gene from *Podospora anserina*, the *aroY* gene from *Enterobacter cloacae* and the *catA* gene from *Candida albicans* (Curran et al., 2013). In a preferred embodiment, the recombinant *Deinococcus* bacterium comprises the *aroZ* gene from *Bacillus thuringiensis* or *Acinetobacter* sp. ADP1, preferably from *Bacillus thuringiensis*, the *aroY* gene from *Klebsiella pneumoniae* and the *catA* gene from *Acinetobacter calcoaceticus*.

In a particular embodiment, the recombinant *Deinococcus* bacterium comprises

- a heterologous nucleic acid sequence encoding a DHS dehydratase selected from the group consisting of DHS dehydratases of SEQ ID NO: 4 and 8, and polypeptides exhibiting DHS dehydratase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, more preferably at least 90 or 95% identity to SEQ ID NO: 4 or 8,

preferably a heterologous nucleic acid sequence encoding the DHS dehydratase of SEQ ID NO: 4 or 8; and

- one or several heterologous nucleic acid sequences encoding a PCA decarboxylase selected from the group consisting of a PCA decarboxylase of SEQ ID NO: 10, 12 and 14 and PCA decarboxylases having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, more preferably at least 90 or 95% identity to SEQ ID NO: 10, 12 or 14, preferably one or several heterologous nucleic acid sequences encoding the PCA decarboxylase of SEQ ID NO: 10, 12 and 14; and

- optionally, a heterologous nucleic acid sequence encoding a catechol 1,2-dioxygenase selected from the group consisting of catechol 1,2-dioxygenases of SEQ ID NO: 25, 29 and 31, and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, more preferably at least 90 or 95% identity to SEQ ID NO: 25, 29 or 31, preferably a heterologous nucleic acid sequence encoding the catechol 1,2-dioxygenase of SEQ ID NO: 25, 29 or 31, preferably of SEQ ID NO: 25.

In another embodiment, the recombinant *Deinococcus* bacterium comprises

- a heterologous nucleic acid sequence encoding a DHS dehydratase selected from the group consisting of DHS dehydratase of SEQ ID NO: 4, and polypeptides exhibiting DHS dehydratase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, more preferably at least 90 or 95% identity to SEQ ID NO: 4, preferably a heterologous nucleic acid sequence encoding the DHS dehydratase of SEQ ID NO: 4; and

- one or several heterologous nucleic acid sequences encoding a PCA decarboxylase selected from the group consisting of PCA decarboxylase of SEQ ID NO: 10, 12 and 14, and PCA decarboxylases having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, more preferably at least 90 or 95% identity to SEQ ID NO: 10, 12 or 14 preferably a heterologous nucleic acid sequence encoding the PCA decarboxylase comprising SEQ ID NO: 10; 12 and 14 and

- optionally, a heterologous nucleic acid sequence encoding a catechol 1,2-dioxygenase selected from the group consisting of catechol 1,2-dioxygenases of SEQ ID NO: 25, 29 and 31, preferably of SEQ ID NO: 25, and polypeptides exhibiting catechol

1,2-dioxygenase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, more preferably at least 90 or 95% identity to SEQ ID NO: 25, 29 or 31, preferably to SEQ ID NO: 25, even more preferably a heterologous nucleic acid sequence encoding the catechol 1,2-dioxygenase of SEQ ID NO: 25, 29 or 31, preferably of SEQ ID NO: 25.

5 In a further embodiment, the recombinant *Deinococcus* bacterium comprises

- a heterologous nucleic acid sequence encoding a DHS dehydratase selected from the group consisting of DHS dehydratase of SEQ ID NO: 8, and polypeptides exhibiting DHS dehydratase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, more preferably at least 90 or 95% identity to SEQ ID NO: 8, preferably a
10 heterologous nucleic acid sequence encoding the DHS dehydratase of SEQ ID NO: 8; and

- one or several heterologous nucleic acid sequences encoding a PCA decarboxylase selected from the group consisting of PCA decarboxylase of SEQ ID NO: 10, 12 and 14, and PCA decarboxylases having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, more preferably at least 90 or 95% identity to SEQ ID NO: 10, 12 or 14
15 preferably a heterologous nucleic acid sequence encoding the PCA decarboxylase comprising SEQ ID NO: 10; 12 and 14 and

- optionally, a heterologous nucleic acid sequence encoding a catechol 1,2-dioxygenase selected from the group consisting of catechol 1,2-dioxygenases of SEQ ID NO: 25, 29 and 31, preferably of SEQ ID NO: 25, and polypeptides exhibiting catechol
20 1,2-dioxygenase activity and having at least 60 %, preferably at least 65, 70, 75, 80 or 85%, more preferably at least 90 or 95% identity to SEQ ID NO: 25, 29 and 31, preferably to SEQ ID NO: 25, even more preferably a heterologous nucleic acid sequence encoding the catechol 1,2-dioxygenase of SEQ ID NO: 25, 29 or 31, preferably of SEQ ID NO: 25.

The recombinant bacterium of the invention may further comprise a heterologous
25 *catX* gene encoding a protein of unknown function but that enhances CatA activity. In the literature, the *catX* gene is also called "orf1" (Neidle and Ornston, 1986). Preferably, the *catX* gene is from *Acinetobacter calcoaceticus*. In *Acinetobacter calcoaceticus*, the *catX* gene lies 1kbp upstream from the *catA* gene.

Nucleic acid sequences encoding heterologous genes may be comprised in one or
30 several expression cassettes. Each expression cassette may comprise *aroZ*, *aroY* and/or

catA genes. In particular, the recombinant *Deinococcus* bacterium of the invention may comprise an expression cassette comprising *aroZ* and *aroY* genes. Preferably, the recombinant *Deinococcus* bacterium of the invention comprises an expression cassette comprising *aroZ*, *aroY* and *catA* genes. These genes may be expressed under the control
5 of a single promoter or under the control of two promoters (with two of these genes under the control of the same promoter). Alternatively, each gene may be expressed under the control of a distinct promoter. These expression cassettes may be integrated into the genome of the bacterium or may be maintained in an episomal form into an expression vector. In embodiments wherein the expression cassette(s) is(are) maintained in an
10 episomal form, the expression vector may be present in the bacterium in one or several copies, depending on the nature of the origin of replication.

Preferably, the expression cassette(s) is(are) integrated into the genome of the bacterium. One or several copies of *aroZ*, *aroY* and/or *catA* genes may be introduced into the genome by methods of recombination, known to the expert in the field, including gene
15 replacement. Preferably, an expression cassette comprising said gene(s) is integrated into the genome. More preferably, an expression cassette comprising *aroZ*, *aroY* and *catA* genes is integrated into the genome. Optionally, additional copies of expression cassettes comprising *aroZ*, *aroY* and/or *catA* genes, preferably *catA* gene, may be further integrated in the genome. The expression cassette(s) may be integrated into the genome in order to
20 inactive target genes. In a particular embodiment, the expression cassette is integrated in the sequence encoding AroE, AroK, AroL, AroA or AroC, preferably in the sequence encoding AroE, in order to block the chorismate pathway. In another particular embodiment, the expression cassette is integrated in a sequence encoding endogenous protocatechuate 3,4-dioxygenase, 3-carboxy-cis,cis-muconate cycloisomerase or 3-
25 oxoadipate enol-lactonase, in order to block the endogenous biosynthetic pathway converting protocatechuate to oxoadipate. Alternatively, or in addition, the expression cassette(s) may be integrated into the genome in a non-coding sequence, e.g. an insertion sequence (IS) (Makarova et al. 2001).

Expression cassettes useful in the present invention comprise at least one gene
30 selected from the group consisting of *aroZ*, *aroY* and *catA* genes, preferably all of them, operably linked to one or more control sequences, typically a transcriptional promoter and a transcription terminator, that direct the expression of said gene(s). In particular,

expression cassettes useful in the present invention may also comprise *aroZ*, *aroY* and *catA* genes, each of them operably linked to one or more control sequences, typically a transcriptional promoter and a transcription terminator, that direct the expression of said genes.

5 The control sequence may include a promoter that is recognized by the host cell. The promoter contains transcriptional control sequences that mediate the expression of the enzyme. The promoter may be any polynucleotide that shows transcriptional activity in the *Deinococcus* bacterium. The promoter may be a native, homologous or heterologous promoter. Preferred promoters are native or homologous. In this regard,
10 various promoters have been studied and used for gene expression in *Deinococcus* bacteria. Examples of suitable promoters include *PtufA* and *PtufB* promoters from the translation elongation factors Tu genes *tufA* (DR0309) and *tufB* (DR2050), the promoter of the *resU* gene located in pI3, the promoter region *PgroESL* of the *groESL* operon (Lecointe *et al*, 2004; Meima *et al*, 2001), or derivatives of such promoters. Preferably,
15 the promoter is a strong constitutive promoter.

The control sequence may also be a transcription terminator, which is recognized by *Deinococcus* bacteria to terminate transcription. The terminator is operably linked to the 3'-terminus of the gene. Any terminator that is functional in *Deinococcus* bacteria may be used in the present invention such as, for example, the terminator term116 described
20 in Lecointe *et al* (2004).

Optionally, the expression cassette may also comprise a selectable marker that permits easy selection of recombinant bacteria. Typically, the selectable marker is a gene encoding antibiotic resistance or conferring autotrophy.

In a particular embodiment, the recombinant *Deinococcus* bacterium of the
25 invention comprises an expression cassette comprising *aroZ* and *aroY*, each of them operably linked to a distinct strong constitutive promoter. Optionally, the bacterium may further comprise another expression cassette comprising *catA* gene operably linked to a strong constitutive promoter.

In another particular embodiment, the recombinant *Deinococcus* bacterium of the invention comprises an expression cassette comprising *aroZ*, *aroY* and *catA* genes operably linked to a strong constitutive promoter.

The expression level of each gene may be also controlled by modulating the
5 strength of the ribosome binding site (RBS) installed in front of the gene.

The *Deinococcus* host cell may be transformed, transfected or transduced in a transient or stable manner. The recombinant *Deinococcus* bacterium of the invention may be obtained by any method known by the skilled person, such as electroporation, conjugation, transduction, competent cell transformation, protoplast transformation,
10 protoplast fusion, biolistic "gene gun" transformation, PEG-mediated transformation, lipid-assisted transformation or transfection, chemically mediated transfection, lithium acetate-mediated transformation or liposome-mediated transformation.

The term "recombinant *Deinococcus* bacterium" also encompasses the genetically modified host cell as well as any progeny that is not identical to the parent host cell, in
15 particular due to mutations that occur during replication.

Inactivation of the endogenous biosynthetic pathway converting DHS to chorismate

In some embodiments, the endogenous biosynthetic pathway converting DHS to chorismate (CHA) is blocked or reduced to improve the flow of carbon towards ccMA.
20 This biosynthetic pathway involves five enzymes, namely AroE, AroK, AroA and AroC. One or several of these enzymatic activities may be inactivated or reduced.

The term "AroE" refers to the enzyme shikimate dehydrogenase (EC 1.1.1.25) encoded by *aroE* gene, that converts DHS to shikimate (SHK). Examples of AroE include, but are not limited to, AroE of *D. geothermalis* (Uniprot accession number: Q1IYW7), *D. radiodurans* (Uniprot accession number: Q9RY73 and Q9RV57), *D. deserti* (Uniprot accession number: C1CUV3), *D. phoenicis* (Uniprot accession number: A0A016QLF8), *D. peraridilitoris* (Uniprot accession number: K9ZXB2), *D. maricopensis* (Uniprot accession number: E8UA95) and *D. proteolyticus* (Uniprot accession number: F0RN73). The gene encoding AroE in the recombinant *Deinococcus*

bacterium of the invention may be easily identified using routine methods, for example based on homology with the nucleic acid encoding any of the above identified AroE enzyme.

The term “AroK” refers to the enzyme shikimate kinase (EC 2.7.1.71) encoded by 5 *aroK* gene. This enzyme converts SHK to 3-phosphoshikimate (S3P). Examples of AroK include, but are not limited to, AroK of *D. geothermalis* (Uniprot accession number: Q1IXK8), *D. radiodurans* (Uniprot accession number: Q9RW93), *D. peraridilitoris* (Uniprot accession number: L0A7T6), *D. maricopenensis* (Uniprot accession number: E8U7Z8), *D. proteolyticus* (Uniprot accession number: F0RLU5), *D. phoenicis* (Uniprot 10 accession number: A0A016QMJ7) and *D. swuensis* (Uniprot accession number: A0A0A7KEP1). The gene encoding AroK in the recombinant *Deinococcus* bacterium of the invention may be easily identified using routine methods, for example based on homology with the nucleic acid encoding any of the above identified AroK enzyme.

The term “AroA” refers to the enzyme 3-phosphoshikimate 1- 15 carboxyvinyltransferase (or 5-enolpyruvylshikimate-3-phosphate synthase or EPSP synthase, EC 2.5.1.19) encoded by *aroA* gene, that converts S3P to 5-enolpyruvylshikimate-3-phosphate (EPSP). Examples of AroA include, but are not limited to, AroA of *D. geothermalis* (Uniprot accession number: Q1IZN3), *D. radiodurans* (Uniprot accession number: Q9RVD3), *D. deserti* (Uniprot accession 20 number: C1D1P6), *D. gobiensis* (Uniprot accession number: H8GWA6), *D. phoenicis* (Uniprot accession number: A0A016QR42), *D. swuensis* (Uniprot accession number: A0A0A7KGT9), *D. peraridilitoris* (Uniprot accession number: L0A580), *D. maricopenensis* (Uniprot accession number: E8U670) and *D. proteolyticus* (Uniprot accession number: F0RN16). The gene encoding AroA in the recombinant *Deinococcus* 25 bacterium of the invention may be easily identified using routine methods, for example based on homology with the nucleic acid encoding any of the above identified AroA enzyme.

The term “AroC” refers to the enzyme chorismate synthase (EC 4.2.3.5) encoded by *aroC* gene, that converts EPSP to chorismate (CHA). Examples of AroC include, but 30 are not limited to, AroC of *D. geothermalis* (Uniprot accession number: Q1IXK9), *D. radiodurans* (Uniprot accession number: Q9RW94), *D. deserti* (Uniprot accession

number: C1CX22), *D. gobiensis* (Uniprot accession number: H8GZ58), *D. phoenicis* (Uniprot accession number: A0A016QND6), *D. peraridilitoris* (Uniprot accession number: L0A5W6), *D. maricopensis* (Uniprot accession number : E8U7Z9), *D. proteolyticus* (Uniprot accession number: F0RLE1) and *D. swuensis* (Uniprot accession number: A0A0A7KJA3). The gene encoding AroC in the recombinant *Deinococcus* bacterium of the invention may be easily identified using routine methods, for example based on homology with the nucleic acid encoding any of the above identified AroC enzyme.

In an embodiment, one or several enzymes of the pathway converting DHS to chorismate are inactivated. *AroE*, *aroK*, *aroA* and/or *aroC* genes may be inactivated by any method known by the skilled person, for example by deletion of all or part of this gene, by introducing a nonsense codon or a mutation inducing a frameshift, or by insertion of an expression cassette, e.g. an expression cassette comprising *aroZ*, *aroY* and/or *catA* genes. In preferred embodiments, all or part of the targeted gene is deleted, for example by gene replacement.

In a particular embodiment, the endogenous biosynthetic pathway converting DHS to chorismate (CHA) is blocked by inactivation of the endogenous *aroE* gene. In a preferred embodiment, all or part of the *aroE* gene is deleted. In a particular embodiment, the *aroE* gene is inactivated by gene replacement or by insertion in said gene of an expression cassette comprising *aroZ*, *AroY* and/or *catA* genes, preferable an expression cassette comprising *aroZ*, *AroY* and *catA* genes.

The inactivation of endogenous biosynthetic pathway converting DHS to chorismate (CHA) has the effect of turning the strain into an auxotroph for the aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and vitamins or vitamin-like intermediates made from the shikimate pathway (p-hydroxy benzoic acid, p- amino benzoic acid, and 2,3-dihydroxy benzoic acid). As a result, this strain during its growth for the production of ccMA requires the exogenous addition of these six compounds (or a common intermediate), thereby adding substantially to the cost of commercial production of ccMA using such a strain.

Thus, in another embodiment, one or several enzymatic activities of the pathway converting DHS to chorismate are reduced. Preferably, this reduction increases the flow

of carbon towards ccMA while maintaining the prototrophy for the aromatic amino acids and vitamins. This reduction may be obtained using altered or “leaky” enzymes or by decreasing the expression level of the genes encoding enzymes of the pathway converting DHS to chorismate.

5 In an embodiment, the recombinant bacterium of the invention comprises a nucleic acid sequence encoding a leaky shikimate dehydrogenase (AroE), i.e. an AroE enzyme that confers prototrophy for the aromatic amino acids and vitamins, but without leading to significant secretion of aromatic compounds. The leaky *aroE* mutant would allow a limited flow of carbon to shikimic acid while accumulating significant amounts
10 of DHS which is then available for the conversion into PCA by the action of an AroZ enzyme. Thus the use of a leaky mutant form of *aroE* would eliminate the dependence on exogenous aromatic amino acids, while still diverting the flow of carbon to ccMA. The leaky AroE enzyme may be an endogenous, homologous or heterologous enzyme. Recombinant *Deinococcus* bacteria comprising a leaky AroE enzyme may be obtained as
15 described in the example 10 of the international patent application WO 2013/116244. The leaky AroE may be in place of the endogenous AroE enzyme. Alternatively, the endogenous *aroE* gene may be inactivated and the leaky AroE expressed from an expression cassette inserted in another locus of the genome or maintained in an episomal form, preferably inserted into the genome.

20 In an alternative embodiment, the recombinant bacterium of the invention comprises AroE, AroK, AroA and/or AroC temperature sensitive (Ts) mutants. Ts mutations are typically missense mutations, which retain the function of a specific essential gene at standard (permissive) low temperature, lack that function at a defined high (non-permissive) temperature, and exhibit partial (hypomorphic) function at an
25 intermediate (semi-permissive) temperature (Ben-Aroya et al., 2010). Culturing the recombinant bacterium of the invention comprising TS mutant AroE, AroK, AroA and/or AroC at semi-permissive temperature thus allows a limited flow of carbon to chorismate and eliminate the dependence on exogenous aromatic amino acids.

 The reduction may also be obtained by decreasing the expression level of one or
30 several enzymes of the chorismate pathway. In particular, the endogenous promoter may be replaced by weaker promoters, such as *PlexA* or *PamyE* promoters (Meima et al.,

2001), thereby inducing a lower expression and thus a decrease of the chorismate production.

Inactivation of the endogenous biosynthetic pathway converting protocatechuate to oxoadipate

5 In some embodiments, the endogenous biosynthetic pathway converting protocatechuate to oxoadipate is blocked or reduced to improve the flow of carbon towards ccMA. This biosynthetic pathway involves three enzymatic activities, namely protocatechuate 3,4-dioxygenase activity, 3-carboxy-cis,cis-muconate cycloisomerase activity and 3-oxoadipate enol-lactonase activity. One or several of these enzymatic
10 activities may be inactivated or reduced.

The enzyme exhibiting protocatechuate 3,4-dioxygenase activity (EC 1.13.11.3) catalyzes the reaction converting 3,4-dihydroxybenzoate (PCA) to 3-carboxy-cis,cis-muconate. In *Deinococcus* bacteria, this enzyme comprises two subunits, i.e. alpha and beta subunits. Examples of *Deinococcus* protocatechuate 3,4-dioxygenases include, but
15 are not limited to, protocatechuate 3,4-dioxygenases of *D. geothermalis* (α -subunit: Uniprot accession number: Q1J3Z7; β -subunit: Uniprot accession number: Q1J3Z6) and *D. deserti* (α -subunit: Uniprot accession number: C1D2D6; β -subunit: Uniprot accession number: C1D2D7). Protocatechuate 3,4-dioxygenase encoding gene may be easily identified in the recombinant *Deinococcus* bacterium of the invention using routine
20 methods, for example based on homology with the nucleic acid encoding any of the above identified protocatechuate 3,4-dioxygenases.

The enzyme exhibiting 3-carboxy-cis,cis-muconate cycloisomerase activity (EC 5.5.1.2) catalyzes the reaction converting 3-carboxy-cis,cis-muconate to γ -carboxymuconolactone. Examples of *Deinococcus* 3-carboxy-cis,cis-muconate
25 cycloisomerases include, but are not limited to, 3-carboxy-cis,cis-muconate cycloisomerases of *D. geothermalis* (Uniprot accession number: Q1J3Z8) and *D. deserti* (Uniprot accession number: C1D2D5). 3-carboxy-cis,cis-muconate cycloisomerase encoding gene may be easily identified in the recombinant *Deinococcus* bacterium of the invention using routine methods, for example based on homology with the nucleic acid
30 encoding any of the above identified 3-carboxy-cis,cis-muconate cycloisomerases.

The enzyme exhibiting 3-oxoadipate enol-lactonase activity (EC 5.5.1.2) catalyzes the reactions converting γ -carboxymuconolactone to 3-oxoadipate-enol-lactone and 3-oxoadipate-enol-lactone to 3-oxoadipate. Examples of *Deinococcus* 3-oxoadipate enol-lactonases include, but are not limited to, 3-oxoadipate enol-lactonases of *D.*
 5 *geothermalis* (Uniprot accession number: Q1J3W1) and *D. deserti* (Uniprot accession number: C1D2D8). 3-oxoadipate enol-lactonase encoding gene may be easily identified in the recombinant *Deinococcus* bacterium of the invention using routine methods, for example based on homology with the nucleic acid encoding any of the above identified 3-oxoadipate enol-lactonases.

10 In an embodiment, one or several enzymes of the pathway converting protocatechuate to oxoadipate are inactivated. Protocatechuate 3,4-dioxygenase, 3-carboxy-cis,cis-muconate cycloisomerase or 3-oxoadipate enol-lactonase encoding gene may be inactivated by any method known by the skilled person, for example by deletion of all or part of this gene, by introducing a nonsense codon or a mutation inducing a
 15 frameshift, or by insertion of an expression cassette, e.g. an expression cassette comprising *aroZ*, *aroY* and/or *catA* genes. In preferred embodiments, all or part of the targeted gene is deleted, for example by gene replacement.

In a particular embodiment, the endogenous biosynthetic pathway converting protocatechuate to oxoadipate is blocked by inactivation of genes encoding
 20 protocatechuate 3,4-dioxygenase, 3-carboxy-cis,cis-muconate cycloisomerase and 3-oxoadipate enol-lactonase.

Enhancement of the flow of carbon through the catechol or muconic acid biosynthetic pathway

To enhance the production of ccMA or catechol, the upper part of the aromatic
 25 amino acid biosynthetic pathway, i.e. the part of the pathway before the conversion of DHS to PCA by the DHS dehydratase, can be modified in order to increase to amount of DHS produced by the bacterium and that can be converted to ccMA or catechol.

In the recombinant *Deinococcus* bacterium of the invention, the activity of one or several enzymes involved in the conversion of phosphoenol pyruvate (PEP) and erythrose

4-phosphate (E4P) to DHS, i.e. AroF, AroG, AroH, AroB and AroD, may be increased compared to the non modified bacterium. Preferably, two, three, four, five, six or seven of these activities are increased. More preferably, all these activities are increased.

The activity of these enzymes may be increased due to the overexpression of their
5 encoding genes. Thus, in an embodiment, at least one gene selected from the group consisting of *aroG*, *aroF*, *aroH*, *aroB* and *aroD* genes, is overexpressed. Preferably, at least two, three, four, five, six or seven of these genes are overexpressed. More preferably, all these genes are overexpressed.

To increase the expression of a gene, the skilled person can use any known
10 techniques such as increasing the copy number of the gene in the bacterium, using a promoter inducing a high level of expression of the gene, i.e. a strong promoter, using elements stabilizing the corresponding messenger RNA or modifying Ribosome Binding Site (RBS) sequences and sequences surrounding them.

In particular, the overexpression may be obtained by increasing the copy number
15 of the gene in the bacterium. One or several copies of the gene may be introduced into the genome by methods of recombination, known to the expert in the field, including gene replacement or multicopy insertion in IS sequences (Makarova et al. 2001). Preferably, an expression cassette comprising the gene is integrated into the genome.

Alternatively, the gene may be carried by an expression vector, preferably a
20 plasmid, comprising an expression cassette with the gene of interest. The expression vector may be present in the bacterium in one or several copies, depending on the nature of the origin of replication.

The overexpression of the gene may also be obtained by using a promoter inducing a high level of expression of the gene. For instance, the promoter of an endogenous gene
25 may be replaced by a stronger promoter, i.e. a promoter inducing a higher level of expression. The promoters suitable to be used in the present invention are known by the skilled person and can be constitutive or inducible, and native, homologous or heterologous.

The overexpressed genes can be native, homologous or heterologous genes.

The terms “AroG”, “AroF” and AroH” refer to DAHP synthases encoded by *aroG*, *aroF* and *aroH* genes, respectively, that convert PEP and E4P to DAHP (Bentley, 1990). The *aroG*, *aroF* or *aroH* gene may be any gene encoding a DAHP synthase, preferably a fungal or bacterial gene, more preferably a bacterial gene. In a particular 5 embodiment, the *aroG*, *aroF* or *aroH* gene is from a *Deinococcus* bacterium. Examples of “AroG”, “AroF” and AroH” from *Deinococcus* bacteria include, but are not limited to, the DAHP synthases of *D. geothermalis* (Uniprot accession numbers: Q1IY17 and Q1IXB8 and NCBI Reference Sequence: WP_039686534.1), *D. radiodurans* (Uniprot accession numbers: Q9RVM6 and Q9RTE8), *D. murrayi* (NCBI Reference Sequence: 10 WP_027459838.1), *D. misasensis* (NCBI Reference Sequence: WP_034334648.1), *D. soli* (NCBI Reference Sequence: WP_046842818.1), *D. maricopenis* (Uniprot accession numbers: E8U3H9, E8U723 and E8U704), *D. marmoris* (NCBI Reference Sequence: WP_029479935.1), *D. deserti* (Uniprot accession numbers: C1CXB8 and C1D0M7, NCBI Reference Sequence: WP_012693957.1), *D. gobiensis* (Uniprot accession 15 numbers: H8GWJ1 and H8GZA8, NCBI Reference Sequence: WP_043800762.1), *D. proteolyticus* (Uniprot accession numbers: F0RP29), *D. peraridilitoris* (Uniprot accession number : K9ZZI4), *D. phoenicis* (Uniprot accession number: A0A016QQD9), *D. swuensis* (Uniprot accession number : A0A0A7KMA6) and *D. aquatilis* (NCBI Reference Sequence: WP_040380876.1). The gene encoding a DAHP synthase in the 20 recombinant *Deinococcus* bacterium of the invention may be easily identified using routine methods, for example based on homology with the nucleic acid encoding any of the above identified enzymes. Any polypeptide, preferably from a *Deinococcus* bacterium, having at least 60%, preferably 80%, more preferably 90%, even more preferably 95% sequence identity to any of the above-identified DAHP synthases may be 25 used.

The term “AroB” refers to the enzyme 3-dehydroquinate synthase (EC 4.2.3.4) encoded by *aroB* gene, that converts DAHP to DHQ. The *aroB* gene may be any gene encoding a 3-dehydroquinate synthase, preferably a fungal or bacterial gene, more preferably a bacterial gene. In a particular embodiment, the *aroB* gene is from a 30 *Deinococcus* bacterium. Examples of “AroB” from *Deinococcus* bacteria include, but are not limited to, the AroB of *D. geothermalis* (Uniprot accession numbers: Q1IXK7 and Q1IJ3M6), *D. radiodurans* (Uniprot accession number: Q9RW92), *D. deserti* (Uniprot

accession number: C1CX24), *D. gobiensis* (Uniprot accession number: H8GZ56), *D. maricopenis* (Uniprot accession number: E8U7Z7), *D. peraridilitoris* (Uniprot accession number: L0A5T3) and *D. swuensis* (Uniprot accession number: A0A0A7KER6). The gene encoding AroB in the recombinant *Deinococcus* bacterium of the invention may be easily identified using routine methods, for example based on homology with the nucleic acid encoding any of the above identified enzymes. Any polypeptide, preferably from a *Deinococcus* bacterium, having at least 60%, preferably 80%, more preferably 90%, even more preferably 95% sequence identity to any of the above-identified AroB enzymes may be used.

10 The term “AroD” or “AroQ” refers to the enzyme 3-dehydroquinate dehydratase (EC 4.2.1.10) encoded by *aroD* (also named *AroQ*) gene, that converts DHQ to DHS. The *aroD* gene may be any gene encoding a 3-dehydroquinate dehydratase, preferably a fungal or bacterial gene, more preferably a bacterial gene. In a particular embodiment, the *aroD* or *AroQ* gene is from a *Deinococcus* bacterium. Examples of “AroD” or “AroQ”
15 from *Deinococcus* bacteria include, but are not limited to, the AroD or AroQ of *D. geothermalis* (Uniprot accession numbers: Q1IXK6 and Q1IXK6), *D. radiodurans* (Uniprot accession number: Q9RW91), *D. deserti* (Uniprot accession number: C1CX25), *D. gobiensis* (Uniprot accession number: H8GZ55), *D. phoenicis* (Uniprot accession number: A0A016QMR8), *D. maricopenis* (Uniprot accession number: E8U7Z6), *D.*
20 *proteolyticus* (Uniprot accession number: F0RLU3), *D. swuensis* (Uniprot accession number: A0A0A7KHD4) and *D. peraridilitoris* (Uniprot accession number: L0A716). The gene encoding AroD in the recombinant *Deinococcus* bacterium of the invention may be easily identified using routine methods, for example based on homology with the nucleic acid encoding any of the above identified enzymes. Any polypeptide, preferably
25 from a *Deinococcus* bacterium, having at least 60%, preferably 80%, more preferably 90%, even more preferably 95% sequence identity to any of the above-identified AroD enzymes may be used.

In a preferred embodiment, at least a gene encoding the DAHP synthase and/or a gene encoding AroB are overexpressed in the recombinant bacterium of the invention.

30 The activity of the enzymes involved in the conversion of PEP and E4P to DHS may also be increased by overexpressing endogenous enzymes, or expressing or

overexpressing improved enzymes, i.e. enzymes that possess at least one mutation in their sequence, in comparison with the amino acid sequence of the wild-type enzyme, said mutation leading to an increase of their activity, an increased specific catalytic activity, an increased specificity for the substrate, an increased protein or RNA stability and/or an increased intracellular concentration of the enzyme, or leading to a feedback resistant mutant. In a particular embodiment, in the recombinant bacterium of the invention, at least one of the enzymes involved in the conversion of PEP and E4P to DHS is an improved enzyme.

In a preferred embodiment, the recombinant bacterium of the invention comprises at least one of the enzymes involved in the conversion of PEP and E4P to DHS which is a feedback resistant mutant. Preferably the recombinant bacterium comprises an AroF, AroG and/or AroH feedback resistant mutant. Indeed, these three proteins are subjected to feedback inhibition by one or more metabolites of shikimic acid pathway responsible for aromatic amino acid biosynthesis. In particular, the endogenous *aroG* gene may be replaced by a modified *aroG* gene which codes for an AroG protein that is resistant to feedback inhibition by one or more metabolites of the aromatic amino acid pathway within the microbial cell, including the aromatic amino acid itself. Feedback resistant mutants of all three enzymes are well known (e.g. Draths et al., 1992; Lutke-Eversloh and Stephanopoulos, 2007; Hu et al, 2003; and Shumilin et al, 1999). Feedback resistant mutants may be mutated endogenous enzymes or homologous or heterologous enzymes. In a more preferred embodiment, AroF, AroG and AroH feedback resistant mutants are overexpressed in the recombinant bacterium of the invention.

In a particular embodiment, the recombinant *Deinococcus* bacterium of the invention expresses a feedback-resistant DAHP synthase. Preferably, the feedback-resistant DAHP synthase is a variant of a *Deinococcus* DAHP synthase. More preferably, the feedback-resistant DAHP synthase is a variant of the *Deinococcus* DAHP synthase set forth in SEQ ID NO: 46 and comprises at least one substitution at position corresponding to residue N13, P156 or S186 of SEQ ID NO: 46. In a more particular embodiment, the feedback-resistant DAHP synthase is a variant of the enzyme set forth in SEQ ID NO: 46 and comprises at least one substitution or combination of substitutions selected from N13K (SEQ ID NO: 47), P156L (SEQ ID NO: 48), S186F (SEQ ID NO:

49), N13K+ P156L (SEQ ID NO: 50), N13K+S186F (SEQ ID NO: 51), P156L +S186F (SEQ ID NO: 52) and N13K+ P156L +S186F (SEQ ID NO: 53).

In some embodiments, in the recombinant *Deinococcus* bacterium of the invention, the activity of one or several enzymes involved in the production of PEP or
5 E4P is increased compared to the non modified bacterium. The activity of these enzymes may be increased due to the overexpression of their encoding genes and/or the use of feedback resistant mutants.

In an embodiment, at least one gene selected from the group consisting of *rpe*, *rpi*, *talB*, *tktA* or *ppsA*, is overexpressed. Preferably, at least two, three, four or five of these
10 genes are overexpressed. More preferably, all these genes are overexpressed. The overexpressed genes can be native, homologous or heterologous genes.

The term “Rpe” refers to ribulose-phosphate 3-epimerase (EC 5.1.3.1) encoded by the *rpe* gene, that converts D-ribulose 5-phosphate to D-xylulose 5-phosphate. The *rpe* gene may be any gene encoding a ribulose-phosphate 3-epimerase, preferably a fungal or
15 bacterial gene, more preferably a bacterial gene. In a particular embodiment, the *rpe* gene is from a *Deinococcus* bacterium. Examples of “Rpe” from *Deinococcus* bacteria include, but are not limited to, the Rpe of *D. geothermalis* (Uniprot accession numbers: Q1IYR9), *D. radiodurans* (Uniprot accession number: Q9RUI5), *D. deserti* (Uniprot accession number: C1D1F4), *D. gobiensis* (Uniprot accession number: H8GVN2), *D. phoenicis*
20 (Uniprot accession number: A0A016QL52), *D. peraridilitoris* (Uniprot accession number: K9ZW42), *D. maricopenensis* (Uniprot accession number: E8UAB1), *D. proteolyticus* (Uniprot accession number: F0RMS8) and *D. swuensis* (Uniprot accession number: A0A0A7KIC0). The gene encoding Rpe in the recombinant *Deinococcus* bacterium of the invention may be easily identified using routine methods, for example
25 based on homology with the nucleic acid encoding any of the above identified enzymes. Any polypeptide, preferably from a *Deinococcus* bacterium, having at least 80%, preferably 90%, more preferably 95% sequence identity to any of the above-identified Rpe enzymes may be used.

The term “Rpi” refers to ribose-5-phosphate isomerase (EC 5.3.1.6) encoded by
30 the *rpi* gene, that converts D-ribose 5-phosphate to D-ribulose 5-phosphate. The *rpi* gene may be any gene encoding a ribose-5-phosphate isomerase, preferably a fungal or

bacterial gene, more preferably a bacterial gene. In a particular embodiment, the *rpi* gene is from a *Deinococcus* bacterium. Examples of “Rpi” from *Deinococcus* bacteria include, but are not limited to, the Rpi of *D. geothermalis* (Uniprot accession numbers: Q1IYX3), *D. radiodurans* (Uniprot accession number: Q9RW24), *D. deserti* (Uniprot accession number: C1CUX0), *D. maricopenis* (Uniprot accession number: E8UAB6), *D. proteolyticus* (Uniprot accession number: F0RLG0), *D. phoenicis* (Uniprot accession number: A0A016QLR1), *D. gobiensis* (Uniprot accession number: H8GT08), *D. peraridilitoris* (Uniprot accession number: K9ZYM6) and *D. swuensis* (Uniprot accession number: A0A0A7KIY3). The gene encoding Rpi in the recombinant *Deinococcus* bacterium of the invention may be easily identified using routine methods, for example based on homology with the nucleic acid encoding any of the above identified enzymes. Any polypeptide, preferably from a *Deinococcus* bacterium, having at least 80%, preferably 90%, more preferably 95% sequence identity to any of the above-identified Rpi enzymes may be used.

The term “TalB” refers to transaldolase (EC 2.2.1.2) encoded by the *talB* gene, that converts sedoheptulose 7-phosphate and D-glyceraldehyde 3-phosphate to D-erythrose 4-phosphate and D-fructose 6-phosphate. The *talB* gene may be any gene encoding a transaldolase, preferably a fungal or bacterial gene, more preferably a bacterial gene. In a particular embodiment, the *talB* gene is from a *Deinococcus* bacterium. Examples of “TalB” from *Deinococcus* bacteria include, but are not limited to, the TalB of *D. geothermalis* (Uniprot accession numbers: Q1IZD4), *D. radiodurans* (Uniprot accession number: Q9RUP6), *D. deserti* (Uniprot accession numbers: C1D3N5 and C1CV54), *D. gobiensis* (Uniprot accession number: H8GUX5), *D. maricopenis* (Uniprot accession number: E8U8S1), *D. proteolyticus* (Uniprot accession number: F0RMW2), *D. phoenicis* (Uniprot accession number: A0A016QNN5) and *D. swuensis* (Uniprot accession number: A0A0A7KDB4). The gene encoding TalB in the recombinant *Deinococcus* bacterium of the invention may be easily identified using routine methods, for example based on homology with the nucleic acid encoding any of the above identified enzymes. Any polypeptide, preferably from a *Deinococcus* bacterium, having at least 80%, preferably 90%, more preferably 95% sequence identity to any of the above-identified TalB enzymes may be used.

The term “TktA” refers to transketolase (EC 2.2.1.1) encoded by the *tktA* gene, that converts sedoheptulose 7-phosphate and D-glyceraldehyde 3-phosphate to D-ribose 5-phosphate and D-xylulose 5-phosphate. The *tktA* gene may be any gene encoding a transketolase, preferably a fungal or bacterial gene, more preferably a bacterial gene. In 5 a particular embodiment, the *tktA* gene is from a *Deinococcus* bacterium. Examples of “TktA” from *Deinococcus* bacteria include, but are not limited to, the TktA of *D. geothermalis* (Uniprot accession numbers: Q1IW07), *D. radiodurans* (Uniprot accession number: Q9RS71), *D. phoenicis* (Uniprot accession numbers: A0A016QSC5), *D. deserti* (Uniprot accession numbers: C1CXQ1), *D. peraridilitoris* (Uniprot accession numbers: 10 K9ZZI7 and L0A0X5), *D. maricopenis* (Uniprot accession number: E8U550) and *D. proteolyticus* (Uniprot accession number: F0RKJ3). The gene encoding TktA in the recombinant *Deinococcus* bacterium of the invention may be easily identified using routine methods, for example based on homology with the nucleic acid encoding any of the above identified enzymes. Any polypeptide, preferably from a *Deinococcus* 15 bacterium, having at least 80%, preferably 90%, more preferably 95% sequence identity to any of the above-identified TktA enzymes may be used.

The term “PpsA” refers to a Phosphoenolpyruvate synthase (EC 2.7.9.2) encoded by the *ppsA* gene, that converts pyruvate to PEP. The *ppsA* gene may be any gene encoding a transketolase, preferably a fungal or bacterial gene, more preferably a bacterial 20 gene. In a particular embodiment, the *ppsA* gene is from a *Deinococcus* bacterium. Examples of “PpsA” from *Deinococcus* bacteria include, but are not limited to, the PpsA of *D. geothermalis* (Uniprot accession numbers: Q1J0R1), *D. radiodurans* (Uniprot accession number: O83026), *D. gobiensis* (Uniprot accession number: H8H237), *D. deserti* (Uniprot accession number: C1CWW0), *D. swuensis* (Uniprot accession number: 25 A0A0A7KM87), *D. phoenicis* (Uniprot accession number: A0A016QMU4) and *D. proteolyticus* (Uniprot accession number: F0RKNK8). The gene encoding PpsA in the recombinant *Deinococcus* bacterium of the invention may be easily identified using routine methods, for example based on homology with the nucleic acid encoding any of the above identified enzymes. Any polypeptide, preferably from a *Deinococcus* 30 bacterium, having at least 80%, preferably 90%, more preferably 95% sequence identity to any of the above-identified PpsA enzymes may be used.

Optionally, in some embodiments, pyruvate kinase I and/or II (EC 2.7.1.40) that use PEP as substrate, may be inactivated or reduced in the recombinant bacterium of the invention. The pyruvate kinase activity may be blocked or reduced as explained above for the enzymes of the chorismate pathway.

- 5 In a particular embodiment, the recombinant *Deinococcus* bacterium of the invention naturally provides sufficient amounts of PEP and E4P and is not genetically modified in order to increase the production of these precursors.

Heterologous catechol-O-methyltransferases

In a further aspect, the present invention also relates to a recombinant
10 *Deinococcus* bacterium comprising (i) a heterologous catechol biosynthetic pathway, i.e. converting 3-dehydroshikimate (DHS) to protocatechuate (PCA) and PCA to catechol, and (ii) a heterologous nucleic acid sequence encoding a polypeptide exhibiting catechol-O-methyltransferase activity, i.e. catalyzing the reaction converting catechol to gaiacol (EC 2.1.1.6). Preferably, said recombinant *Deinococcus* bacterium does not exhibit any
15 catechol 1,2 dioxygenase activity.

The polypeptide exhibiting catechol-O-methyltransferase activity may be any known catechol-O-methyltransferase (COMT). Preferably, the polypeptide exhibiting COMT activity is selected from the group consisting of COMT from *Mycobacterium vanbaalenii* (Uniprot accession number: A1TA78; SEQ ID NO: 54) and any polypeptide
20 exhibiting COMT activity and having at least 60 %, preferably at least 65, 70, 75, 80, 85, 90 or 95%, identity to said COMT. More preferably, the polypeptide exhibiting COMT activity is COMT from *Mycobacterium vanbaalenii* (SEQ ID NO: 54). An example of nucleic acid sequence encoding said COMT is set forth in SEQ ID NO: 55.

Due to the temperature sensitivity of gaiacol, the recombinant *Deinococcus*
25 bacterium expressing a heterologous COMT is preferably a mesophilic strain, more preferably *D. grandis*, *D. cellulolysiticus*, *D. depolymerans*, *D. aquaticus*, *D. deserti*, *D. wulumuqiensis*, *D. proteolyticus*, *D. gobiensis misasensis*, *D. frigans*, *D. marmoris*, *D. ficus*, *D. apachensis*, *D. aquatilis*, *D. pimensis*, *D. peraridilitoris*, *D. puniceus*,

D.phoenicis, *D. swuensis* or *D. actinosclerus*, and even more preferably *D. depolymerans* or *D. aquaticus*.

Nucleic acid sequences encoding heterologous genes may be comprised in one or several expression cassettes. Each expression cassette may comprise *aroZ*, *aroY* and/or
5 *COMT* genes. In particular, the recombinant *Deinococcus* bacterium of the invention may comprise (i) an expression cassette comprising *aroZ* gene, an expression cassette comprising *aroY* gene and an expression cassette comprising *COMT* gene; (ii) an expression cassette comprising *aroZ* and *aroY* genes and an expression cassette comprising *COMT* gene; (iii) an expression cassette comprising *aroZ* and *COMT* genes
10 and an expression cassette comprising *aroY* gene; (iv) an expression cassette comprising *aroY* and *COMT* genes and an expression cassette comprising *aroZ* gene, or (v) an expression cassette comprising *aroZ*, *aroY* and *COMT* genes.

Preferably, the recombinant *Deinococcus* bacterium of the invention comprises an expression cassette comprising *aroZ*, *aroY* and *COMT* genes. These genes may be
15 expressed under the control of a single promoter or under the control of two promoters (with two of these genes under the control of the same promoter). Alternatively, each gene may be expressed under the control of a distinct promoter. These expression cassettes may be integrated into the genome of the bacterium or may be maintained in an episomal form into an expression vector. In embodiments wherein the expression
20 cassette(s) is(are) maintained in an episomal form, the expression vector may be present in the bacterium in one or several copies, depending on the nature of the origin of replication.

Preferably, the expression cassette(s) is(are) integrated into the genome of the bacterium. One or several copies of *aroZ*, *aroY* and/or *COMT* genes may be introduced
25 into the genome by methods of recombination, known to the expert in the field, including gene replacement. Preferably, an expression cassette comprising said gene(s) is integrated into the genome. More preferably, an expression cassette comprising *aroZ*, *aroY* and *COMT* genes is integrated into the genome. Optionally, additional copies of expression cassettes comprising *aroZ*, *aroY* and/or *COMT* genes may be further integrated in the
30 genome. The expression cassette(s) may be integrated into the genome in order to inactive target genes. In a particular embodiment, the expression cassette is integrated in the

sequence encoding AroE, AroK, AroL, AroA or AroC, preferably in the sequence encoding AroE, in order to block the chorismate pathway. In another particular embodiment, the expression cassette is integrated in a sequence encoding endogenous protocatechuate 3,4-dioxygenase, 3-carboxy-cis,cis-muconate cycloisomerase or 3-oxoadipate enol-lactonase, in order to block the endogenous biosynthetic pathway converting protocatechuate to oxoadipate. Alternatively, or in addition, the expression cassette(s) may be integrated into the genome in a non-coding sequence, e.g. an insertion sequence (IS) (Makarova et al. 2001).

The recombinant *Deinococcus* bacterium comprising (i) a heterologous catechol biosynthetic pathway, i.e. converting 3-dehydroshikimate (DHS) to protocatechuate (PCA) and PCA to catechol, and (ii) a heterologous nucleic acid sequence encoding a polypeptide exhibiting catechol-O-methyltransferase activity, may further comprise an enhancement of the carbon flow to catechol production i.e. the upper part of the aromatic amino acid biosynthetic pathway before the conversion of DHS to PCA as described above. Optionally, the endogenous biosynthetic pathway converting protocatechuate to oxoadipate and/or the endogenous biosynthetic pathway converting DHS to chorismate may be inactivated.

Cell extract

In another aspect, the present invention also relates to a cell extract of the recombinant *Deinococcus* bacterium of the invention. As used herein, the term “cell extract” refers to any fraction obtained from a host cell, such as a cell supernatant, a cell debris, cell walls, DNA extract, enzymes or enzyme preparation or any preparation derived from host cells by chemical, physical and/or enzymatic treatment, which is essentially or mainly free of living cells such as whole broken cell extract.

Methods of production

In a further aspect, the present invention relates to a use of a recombinant *Deinococcus* bacterium of the invention for producing ccMA.

In particular, the present invention relates to a method of producing ccMA comprising (i) culturing a recombinant *Deinococcus* bacterium according to the invention comprising a heterologous muconic acid biosynthetic pathway under conditions suitable to produce ccMA and optionally (ii) recovering said ccMA.

5 The present invention also relates to a method of producing catechol comprising (i) culturing a recombinant *Deinococcus* bacterium according to the invention comprising a heterologous catechol biosynthetic pathway under conditions suitable to produce catechol, and optionally (ii) recovering said catechol.

The present invention further relates to a method of producing ccMA comprising
10 (i) culturing a recombinant *Deinococcus* bacterium according to the invention comprising a heterologous catechol biosynthetic pathway under conditions suitable to produce catechol, (ii) enzymatically converting catechol to ccMA, and optionally (iii) recovering said ccMA. Optionally, catechol produced in step (i) may be recovered before enzymatic conversion. Catechol produced by the recombinant bacterium of the invention is secreted
15 in the culture supernatant. The enzymatic conversion of catechol to ccMA may be carried out using any enzyme exhibiting catechol 1,2-dioxygenase, in particular CatA enzymes disclosed above. This enzyme may be provided by another strain co-cultured with the recombinant *Deinococcus* bacterium of the invention and secreting CatA enzyme. Alternatively, the enzyme may be added to the culture medium or reaction medium
20 comprising the catechol. The enzyme may be purified or may be comprised in a cell extract, in particular a cell extract of the recombinant *Deinococcus* bacterium of the invention exhibiting CatA activity.

The present invention further relates to a method of producing gaiacol comprising (i) culturing a recombinant *Deinococcus* bacterium according to the invention comprising
25 a heterologous catechol biosynthetic pathway and a heterologous nucleic acid sequence encoding a polypeptide exhibiting catechol-O-methyltransferase activity, under conditions suitable to produce gaiacol, and optionally (ii) recovering said gaiacol.

The present invention further relates to a method of producing vanillin comprising (i) culturing a recombinant *Deinococcus* bacterium according to the invention comprising
30 a heterologous catechol biosynthetic pathway and a heterologous nucleic acid sequence encoding a polypeptide exhibiting catechol-O-methyltransferase activity, under

conditions suitable to produce gaiacol, (ii) converting gaiacol to vanillin and, optionally (iii) recovering said vanillin. Optionally, gaiacol produced in step (i) may be recovered before conversion. The conversion of gaiacol to vanillin may be carried out using any method known by the skilled person, e.g. the method described by Mottern (Mottern, 5 1934).

Conditions suitable to produce ccMA, catechol or gaiacol may be easily determined by the skilled person according to the recombinant *Deinococcus* bacterium used. In particular, the carbon source may be selected from the group consisting of C5 sugars such as xylose and arabinose, C6 sugars such as glucose, cellobiose, saccharose 10 and starch.

Preferably, ccMA, catechol or gaiacol is produced from renewable, biologically derived carbon sources. In particular, when the recombinant *Deinococcus* bacterium exhibits cellulolytic and/or xylanolytic activity, more complex carbon sources can be used such as cellulosic biomass. As used herein, the term “cellulosic biomass” refers to any 15 biomass material, preferably vegetal biomass, comprising cellulose, hemicellulose and/or lignocellulose, preferably comprising cellulose and hemicellulose. Cellulosic biomass includes, but is not limited to, plant material such as forestry products, woody feedstock (softwoods and hardwoods), agricultural wastes and plant residues (such as corn stover, sorghum, sugarcane bagasse, grasses, rice straw, wheat straw, empty fruit bunch from 20 oil palm and date palm, agave bagasse, from tequila industry), perennial grasses (switchgrass, miscanthus, canary grass, erianthus, napier grass, giant reed, and alfalfa); municipal solid waste (MSW), aquatic products such as algae and seaweed, wastepaper, leather, cotton, hemp, natural rubber products, and food and feed processing by-products.

Preferably, if the cellulosic biomass comprises lignocellulose, this biomass is pre- 25 treated before hydrolysis. This pretreatment is intended to open the bundles of lignocelluloses in order to access the polymer chains of cellulose and hemicellulose. Pretreatment methods are well known by the skilled person and may include physical pretreatments (e.g. high pressure steaming, extrusion, pyrolysis or irradiation), physicochemical and chemical pretreatments (e.g. ammonia fiber explosion, treatments 30 with alkaline, acidic, solvent or oxidizing agents) and/or biological pretreatments.

Temperature conditions can also be adapted depending on the use of mesophilic or thermophilic *Deinococcus* bacteria.

In an embodiment, the *Deinococcus* bacterium is a thermophilic *Deinococcus*, such as for example *D. geothermalis*, and the culture of the recombinant *Deinococcus* 5 bacterium under conditions suitable to produce ccMA, catechol or gaiacol is performed at a temperature comprised between 35 and 60°C, preferably 37 and 55°C, more preferably at about 48°C.

In another embodiment, the *Deinococcus* bacterium is a mesophilic *Deinococcus* and the culture of the recombinant *Deinococcus* bacterium under conditions suitable to 10 produce ccMA, catechol or gaiacol is performed at a temperature comprised between 30 and 42°C, preferably between 35 and 40°C, more preferably at about 37°C.

In a further aspect, the present invention relates to a use of a recombinant *Deinococcus* bacterium of the invention for producing *cis-trans* and/or *trans-trans* muconic acid (ctMA and ttMA, respectively). The present invention also relates to a 15 method of producing ctMA and/or ttMA comprising (i) producing ccMA according to the method of the invention and as described above and (ii) isomerizing said ccMA to produce ctMA and/or ttMA, and (iii) optionally recovering said ctMA and/or ttMA. The method may further comprise an additional step of recovering ccMA before isomerization. Optionally, ccMA may be isomerized to produce ctMA and ctMA may be 20 further isomerized to produce ttMA. The isomerization may be carried out by any method known by the skilled person such as for example the method disclosed in the US patents 8,809,583, 8,426,639 or 8,367,858.

In a further aspect, the present invention relates to a use of a recombinant *Deinococcus* bacterium of the invention for producing adipic acid. The present invention 25 also relates to a method of producing adipic acid comprising (i) producing ccMA according to the method of the invention and as described above and (ii) reducing said ccMA to produce adipic acid, and (iii) optionally recovering said adipic acid. The method may further comprise an additional step of recovering ccMA before reduction. The reduction may be carried out by any method known by the skilled person such as for 30 example the method disclosed in Niu et al., 2002.

In a further aspect, the present invention relates to a use of a recombinant *Deinococcus* bacterium of the invention for producing teraphthalic acid and/or trimellititic acid. The present invention also relates to a method of producing terephthalic acid and/or trimellitic acid comprising (i) producing ccMA, ctMA and/or ttMA according to the
5 method of the invention and as described above and (ii) converting said ccMA, ctMA and/or ttMA to produce terephthalic acid and/or trimellitic acid, and (iii) optionally recovering said terephthalic acid and/or trimellitic acid. The method may further comprise an additional step of recovering ccMA, ctMA and/or ttMA before conversion. The conversion of ccMA, ctMA and/or ttMA to teraphthalic acid and/or trimellititic acid may
10 be carried out by any method known by the skilled person such as for example the method disclosed in the US patent 8,367,858.

Muconic acid, adipic acid, teraphthalic acid or trimellititic acid produced according to the methods of the invention may be further used in the production various compounds such as, for example, Nylon 6, Nylon-6,6, polyester polyols, polytrimethylene
15 terephthalate, polyethylene terephthalate, dimethyl terephthalate, trimellitic anhydride, industrial plastis, resins, food ingredients, plasticizers, cosmetics, pharmaceuticals or other polymers.

The methods of the invention may be performed in a reactor, in particular a reactor of conversion of biomass. By "reactor" is meant a conventional fermentation tank or any
20 apparatus or system for biomass conversion, typically selected from bioreactors, biofilters, rotary biological contactors, and other gaseous and/or liquid phase bioreactors. The apparatus which can be used according to the invention can be used continuously or in batch loads. Depending on the cells used, the method may be conducted under aerobiosis, anaerobiosis or microaerobiosis.

25

The following items are herein described:

1. A recombinant *Deinococcus* bacterium expressing a heterologous polypeptide exhibiting 3-dehydroshikimate dehydratase activity and a heterologous polypeptide exhibiting protocatechuate decarboxylase activity.

2. The recombinant bacterium of item 1, wherein said bacterium further expresses a heterologous polypeptide exhibiting catechol 1,2-dioxygenase activity.

3. The recombinant bacterium according to item 1 or 2, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratases from *Bacillus thuringiensis*, *Podospira anserina*, , *Klebsiella pneumoniae*, *Acinetobacter calcoaceticus*, *Acinetobacter sp. ADP1*, *Acinetobacter baylyi*, *Neurospora crassa*, *Aspergillus nidulans*, *Gluconobacter oxydans* and *Pseudomonas putida*, in particular *Pseudomonas putida* KT2440 and *Pseudomonas putida* H8234.

10 4. The recombinant bacterium according to any of items 1 to 3, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratases from *Bacillus thuringiensis*, *Podospira anserina*, *Pseudomonas putida* and *Acinetobacter sp. ADP1*, preferably from *Bacillus thuringiensis*, *Podospira anserina* and *Acinetobacter sp. ADP1*, and more
15 preferably from *Bacillus thuringiensis* and *Acinetobacter sp. ADP1*.

5. The recombinant bacterium according to any of items 1 to 4, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratases of SEQ ID NO: 4, 2, 6 and 8, and polypeptides exhibiting 3-dehydroshikimate dehydratase activity and having at least 60
20 % identity to SEQ ID NO: 4, 2, 6 or 8.

6. The recombinant bacterium according to any of items 1 to 5, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratases of SEQ ID NO: 4 and 8, and polypeptides exhibiting 3-dehydroshikimate dehydratase activity and having at least 60
25 % identity to SEQ ID NO: 4 or 8.

7. The recombinant bacterium according to any of items 1 to 6, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratase of SEQ ID NO: 4, and polypeptides exhibiting 3-dehydroshikimate dehydratase activity and having at least 60 % identity to
30 SEQ ID NO: 4.

8. The recombinant bacterium according to any of items 1 to 7, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratase of SEQ ID NO: 4, and polypeptides exhibiting 3-dehydroshikimate dehydratase activity and having at least 80 % identity to
5 SEQ ID NO: 4, preferably at least 90 % identity to SEQ ID NO: 4.

9. The recombinant bacterium according to any of items 1 to 8, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is a 3-dehydroshikimate dehydratase of SEQ ID NO: 4.

10. The recombinant bacterium according to any of items 1 to 6, wherein said
10 polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratase of SEQ ID NO: 8, and polypeptides exhibiting 3-dehydroshikimate dehydratase activity and having at least 60 % identity to SEQ ID NO: 8.

11. The recombinant bacterium according to any of items 1 to 6, wherein said
15 polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratase of SEQ ID NO: 8, and polypeptides exhibiting 3-dehydroshikimate dehydratase activity and having at least 80 % identity to SEQ ID NO: 8, preferably at least 90 % identity to SEQ ID NO: 8.

12. The recombinant bacterium according to any of items 1 to 6, wherein said
20 polypeptide exhibiting 3-dehydroshikimate dehydratase activity is a 3-dehydroshikimate dehydratase of SEQ ID NO: 8.

13. The recombinant bacterium according to any of items 1 to 5, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratase of SEQ ID NO: 2, and polypeptides
25 exhibiting 3-dehydroshikimate dehydratase activity and having at least 60 % identity to SEQ ID NO: 2.

14. The recombinant bacterium according to any of items 1 to 5, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratase of SEQ ID NO: 2, and polypeptides

exhibiting 3-dehydroshikimate dehydratase activity and having at least 80 % identity to SEQ ID NO: 2, preferably at least 90 % identity to SEQ ID NO: 2.

15. The recombinant bacterium according to any of items 1 to 5, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is a 3-dehydroshikimate dehydratase of SEQ ID NO: 2.

16. The recombinant bacterium according to any of items 1 to 5, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratase of SEQ ID NO: 6, and polypeptides exhibiting 3-dehydroshikimate dehydratase activity and having at least 60 % identity to SEQ ID NO: 6.

17. The recombinant bacterium according to any of items 1 to 5, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratase of SEQ ID NO: 6, and polypeptides exhibiting 3-dehydroshikimate dehydratase activity and having at least 80 % identity to SEQ ID NO: 6, preferably at least 90 % identity to SEQ ID NO: 6.

18. The recombinant bacterium according to any of items 1 to 5, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is a 3-dehydroshikimate dehydratase of SEQ ID NO: 6.

19. The recombinant bacterium according to any of items 1 to 18, wherein said polypeptide exhibiting protocatechuate decarboxylase activity is selected from the group consisting of 3-protocatechuate decarboxylases from *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Sedimentibacter hydroxybenzoicus*, preferably from *Klebsiella pneumoniae*.

20. The recombinant bacterium according to any of items 1 to 19, wherein said polypeptide exhibiting protocatechuate decarboxylase activity is selected from the group consisting of (i) a PCA decarboxylase comprising polypeptides of SEQ ID NO: 10, 12 and 14, (ii) a PCA decarboxylase comprising polypeptides of SEQ ID NO: 17, 19 and 21, (iii) the PCA decarboxylase of SEQ ID NO: 23, and (iv) PCA decarboxylases having at least 60 % identity to SEQ ID NO: 10, 12, 14, 17, 19, 21 or 23.

21. The recombinant bacterium according to any of items 1 to 20, wherein said polypeptide exhibiting protocatechuate decarboxylase activity is selected from the group consisting of a PCA decarboxylase comprising polypeptides of SEQ ID NO: 10, 12 and 14, and PCA decarboxylases having at least 60 % identity to SEQ ID NO: 10, 12 or 14.
- 5 22. The recombinant bacterium according to any of items 1 to 20, wherein said polypeptide exhibiting protocatechuate decarboxylase activity is selected from the group consisting of a PCA decarboxylase comprising polypeptides of SEQ ID NO: 10, 12 and 14, and PCA decarboxylases having at least 80 % identity to SEQ ID NO: 10, 12 or 14, preferably at least 90 % identity to SEQ ID NO: 10, 12 or 14.
- 10 23. The recombinant bacterium according to any of items 1 to 20, wherein said polypeptide exhibiting protocatechuate decarboxylase activity is a PCA decarboxylase comprising polypeptides of SEQ ID NO: 10, 12 and 14.
24. The recombinant bacterium according to any of items 1 to 20, wherein said polypeptide exhibiting protocatechuate decarboxylase activity is selected from the group
15 consisting of a PCA decarboxylase comprising polypeptides of SEQ ID NO: 17, 19 and 21, and PCA decarboxylases having at least 60 % identity to SEQ ID NO: 17, 19 or 21.
25. The recombinant bacterium according to any of items 1 to 20, wherein said polypeptide exhibiting protocatechuate decarboxylase activity is selected from the group consisting of a PCA decarboxylase comprising polypeptides of SEQ ID NO: 17, 19 and
20 21, and PCA decarboxylases having at least 80 % identity to SEQ ID NO: 17, 19 or 21, preferably at least 90 % identity to SEQ ID NO: 17, 19 or 21.
26. The recombinant bacterium according to any of items 1 to 20, wherein said polypeptide exhibiting protocatechuate decarboxylase activity is a PCA decarboxylase comprising polypeptides of SEQ ID NO: 17, 19 and 21.
- 25 27. The recombinant bacterium according to any of items 1 to 20, wherein said polypeptide exhibiting protocatechuate decarboxylase activity is selected from the group consisting of a PCA decarboxylase of SEQ ID NO: 23, and PCA decarboxylases having at least 60 % identity to SEQ ID NO: 23.

25. The recombinant bacterium according to any of items 1 to 20, wherein said polypeptide exhibiting protocatechuate decarboxylase activity is selected from the group consisting of a PCA decarboxylase of SEQ ID NO: 23, and PCA decarboxylases having at least 80 % identity to SEQ ID NO: 23, preferably at least 90 % identity to SEQ ID NO: 23.

26. The recombinant bacterium according to any of items 1 to 20, wherein said polypeptide exhibiting protocatechuate decarboxylase activity is a PCA decarboxylase of SEQ ID NO: 23.

27. The recombinant bacterium according to any of items 2 to 26, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenases from *Acinetobacter radioresistens*, *Acinetobacter calcoaceticus*, *Candida albicans*, *Burkholderia mallei*, *Burkholderia xenovorans*, *Pseudomonas putida*, *Stenotrophomonas maltophilia* KB2, *Cupriavidus metallidurans* CH34, *Burkholderia* sp. TH2, *Rhodococcus opacus*, *Rhodococcus erythropolis* and *Acinetobacter* sp. ADP1.

28. The recombinant bacterium according to any of items 2 to 27, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenases from *Acinetobacter radioresistens*, *Acinetobacter calcoaceticus*, *Candida albicans*, *Burkholderia mallei*, *Burkholderia xenovorans* and *Pseudomonas putida*, preferably from *Acinetobacter calcoaceticus*, *Candida albicans* and *Burkholderia xenovorans*.

29. The recombinant bacterium according to any of items 2 to 27, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenases of SEQ ID NO: 25, 27, 29, 31, 33, 34, 35, 36, 37, 38, 39, 40 and 41 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 25, 27, 29, 31, 33, 34, 35, 36, 37, 38, 39, 40 or 41.

30. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenases of SEQ ID NO: 25, 29 and 31 and polypeptides

exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 25, 29 or 31.

31. The recombinant bacterium according to any of items 2 to 30, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group
5 consisting of catechol 1,2-dioxygenase of SEQ ID NO: 25 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 25.

32. The recombinant bacterium according to any of items 2 to 31, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 25 and polypeptides exhibiting
10 catechol 1,2-dioxygenase activity and having at least 80 % identity to SEQ ID NO: 25, preferably at least 90 % identity to SEQ ID NO: 25.

33. The recombinant bacterium according to any of items 2 to 32, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is a catechol 1,2-dioxygenase of SEQ ID NO: 25.

15 34. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 27 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 27.

35. The recombinant bacterium according to any of items 2 to 29, wherein said
20 polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 27 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 80 % identity to SEQ ID NO: 27, preferably at least 90 % identity to SEQ ID NO: 27.

36. The recombinant bacterium according to any of items 2 to 29, wherein said
25 polypeptide exhibiting catechol 1,2-dioxygenase activity is a catechol 1,2-dioxygenase of SEQ ID NO: 27.

37. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group

consisting of catechol 1,2-dioxygenase of SEQ ID NO: 29 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 29.

38. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group
5 consisting of catechol 1,2-dioxygenase of SEQ ID NO: 29 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 80 % identity to SEQ ID NO: 29, preferably at least 90 % identity to SEQ ID NO: 29.

39. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is a catechol 1,2-dioxygenase of
10 SEQ ID NO: 29.

40. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 31 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 31.

15 41. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 31 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 80 % identity to SEQ ID NO: 31, preferably at least 90 % identity to SEQ ID NO: 31.

20 42. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is a catechol 1,2-dioxygenase of SEQ ID NO: 31.

43. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group
25 consisting of catechol 1,2-dioxygenase of SEQ ID NO: 33 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 33.

44. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 33 and polypeptides exhibiting

catechol 1,2-dioxygenase activity and having at least 80 % identity to SEQ ID NO: 33, preferably at least 90 % identity to SEQ ID NO: 33.

48. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is a catechol 1,2-dioxygenase of
5 SEQ ID NO: 33.

49. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 34 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 34.

10 50. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 34 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 80 % identity to SEQ ID NO: 34, preferably at least 90 % identity to SEQ ID NO: 34.

15 51. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is a catechol 1,2-dioxygenase of SEQ ID NO: 34.

52. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group
20 consisting of catechol 1,2-dioxygenase of SEQ ID NO: 35 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 35.

53. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 35 and polypeptides exhibiting
25 catechol 1,2-dioxygenase activity and having at least 80 % identity to SEQ ID NO: 35, preferably at least 90 % identity to SEQ ID NO: 35.

54. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is a catechol 1,2-dioxygenase of SEQ ID NO: 35.

55. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 36 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 36.

5 56. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 36 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 80 % identity to SEQ ID NO: 36, preferably at least 90 % identity to SEQ ID NO: 36.

10 57. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is a catechol 1,2-dioxygenase of SEQ ID NO: 36.

58. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group
15 consisting of catechol 1,2-dioxygenase of SEQ ID NO: 37 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 37.

59. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 37 and polypeptides exhibiting
20 catechol 1,2-dioxygenase activity and having at least 80 % identity to SEQ ID NO: 37, preferably at least 90 % identity to SEQ ID NO: 37.

60. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is a catechol 1,2-dioxygenase of SEQ ID NO: 37.

25 61. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 38 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 38.

62. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 38 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 80 % identity to SEQ ID NO: 38,
5 preferably at least 90 % identity to SEQ ID NO: 38.

63. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is a catechol 1,2-dioxygenase of SEQ ID NO: 38.

64. The recombinant bacterium according to any of items 2 to 29, wherein said
10 polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 39 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 39.

65. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group
15 consisting of catechol 1,2-dioxygenase of SEQ ID NO: 39 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 80 % identity to SEQ ID NO: 39, preferably at least 90 % identity to SEQ ID NO: 39.

66. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is a catechol 1,2-dioxygenase of
20 SEQ ID NO: 39.

67. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 40 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 40.

25 68. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 40 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 80 % identity to SEQ ID NO: 40, preferably at least 90 % identity to SEQ ID NO: 40.

69. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is a catechol 1,2-dioxygenase of SEQ ID NO: 40.

70. The recombinant bacterium according to any of items 2 to 29, wherein said
5 polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 41 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 41.

71. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group
10 consisting of catechol 1,2-dioxygenase of SEQ ID NO: 41 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 80 % identity to SEQ ID NO: 41, preferably at least 90 % identity to SEQ ID NO: 41.

72. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is a catechol 1,2-dioxygenase of
15 SEQ ID NO: 41.

73. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 41 and a variant thereof comprising at least one substitution at position corresponding to residue G72, L73 or P76,
20 preferably at least one substitution or combination of substitutions selected from G72A, L73F, P76A, G72A+L73F, G72A+P76A, P76A+L73F and G72A+L72F+P76A.

74. The recombinant bacterium according to any of items 2 to 29, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is a variant of the catechol 1,2-dioxygenase of SEQ ID NO: 41 comprising at least one substitution or combination of
25 substitutions selected from G72A, P76A, L73F and L73F+P76A.

75. The recombinant bacterium according to any of items 1 to 74, wherein the endogenous biosynthetic pathway converting 3-dehydroshikimate to chorismate is blocked or reduced.

76. The recombinant bacterium according to item 75, wherein the endogenous gene encoding shikimate dehydrogenase (AroE) is inactivated.

77. The recombinant bacterium according to item 75 or 76, wherein the endogenous shikimate dehydrogenase activity is reduced.

5 78. The recombinant bacterium according to any of items 1 to 77, wherein the endogenous biosynthetic pathway converting protocatechuate to oxoadipate is blocked or reduced.

79. The recombinant bacterium according to item 78, wherein the endogenous biosynthetic pathway converting protocatechuate to oxoadipate is blocked by inactivation
10 one or several endogenous genes selected from genes encoding protocatechuate 3,4-dioxygenase, 3-carboxy-cis,cis-muconate cycloisomerase and 3-oxoadipate enol-lactonase.

80. The recombinant bacterium according to any of items 1 to 79, wherein one or several of the enzymes involved in the conversion of phosphoenolpyruvate and erythrose
15 4-phosphate to 3-dihydroshikimate, preferably selected from AroF, AroG, AroH, AroB and AroD, are overexpressed and/or are feedback inhibition resistant enzymes.

81. The recombinant bacterium according to item 80, wherein the recombinant bacterium expresses a feedback inhibition resistant DAHP synthase.

82. The recombinant bacterium according to item 81, wherein the feedback
20 inhibition resistant DAHP synthase is a variant of the *Deinococcus* DAHP synthase set forth in SEQ ID NO: 46 and comprises at least one substitution at position corresponding to residue N13, P156 or S186 of SEQ ID NO: 46, preferably selected from N13K, P156L, S186F, N13K+ P156L, N13K+S186F, P156L +S186F and N13K+ P156L +S186F.

83. The recombinant bacterium of any of items 1 to 82, further expresses a
25 heterologous polypeptide exhibiting catechol-O-methyltransferase activity.

84. The recombinant bacterium of item 83, wherein the polypeptide exhibiting catechol-O-methyltransferase activity is selected from the group consisting of COMT from *Mycobacterium vanbaalenii* (SEQ ID NO: 54) and any polypeptide exhibiting COMT activity and having at least 60 % identity to SEQ ID NO: 54.

85. A method of producing *cis-cis* muconic acid comprising culturing a recombinant *Deinococcus* bacterium according to any of items 2 to 82 under conditions suitable to produce *cis-cis* muconic acid, and optionally recovering said *cis-cis* muconic acid.

5 86. A method of producing catechol comprising culturing a recombinant *Deinococcus* bacterium according to any of items 1 to 82 under conditions suitable to produce catechol, and optionally recovering said catechol.

87. A method of producing *cis-cis* muconic acid comprising (i) producing catechol according to the method of item 86, (ii) enzymatically converting catechol to *cis-cis*
10 muconic acid, and optionally (iii) recovering said *cis-cis* muconic acid.

88. The method according to any of items 85 to 87 wherein the culture of the recombinant *Deinococcus* bacterium under conditions suitable to produce *cis-cis* muconic acid or catechol is performed at a temperature comprised between 37 and 55°C.

89. A method of producing adipic acid comprising producing *cis-cis* muconic acid
15 according to the method of item 85, 87 or 88 and reducing said *cis-cis* muconic acid to produce adipic acid, and optionally recovering said adipic acid.

90. A method of producing *cis-trans* and/or *trans-trans* muconic acid comprising producing *cis-cis* muconic acid according to the method of item 85, 87 or 88 and isomerizing said *cis-cis* muconic acid to produce *cis-trans* and/or *trans-trans* muconic
20 acid, and optionally recovering said *cis-trans* and/or *trans-trans* muconic acid.

91. A method of producing gaiacol comprising (i) culturing a recombinant *Deinococcus* bacterium according to item 83 or 84 under conditions suitable to produce gaiacol, and optionally (ii) recovering said gaiacol.

92. A method of producing vanillin comprising (i) culturing a recombinant
25 *Deinococcus* bacterium according to item 83 or 84 under conditions suitable to produce gaiacol, (ii) converting gaiacol to vanillin and, optionally (iii) recovering said vanillin.

93. The method of item 92, further comprising recovering gaiacol produced in step (i) before conversion.

Further aspects and advantages of the present invention will be described in the following examples, which should be regarded as illustrative and not limiting.

EXAMPLES

Example 1: Catechol production by recombinant *Deinococcus geothermalis*

5 *Materiel & Methods:*

A *Deinococcus geothermalis* strain was genetically engineered to produce catechol.

Genomic DNAs were prepared using Dneasy&Blood QIAGEN Kit as indicated by the Manufacturer. Each gene was amplified and assembled by overlapping PCR.
10 Insertion of DNA fragments into the chromosome of *D. geothermalis* was performed using homologous recombination mechanism. Insertion cassettes comprised a nucleic acid sequence to be inserted into the chromosome flanked by 1500 bp region homologous to the sequence upstream or downstream the chromosomal target *aroE* gene.

For the expression of heterologous genes, strong constitutive promoters were used
15 such as PtufA and PtufB promoters from the translation elongation factors Tu genes *tufA* (DR0309) and *tufB* (DR2050), or the promoter region PgroESL of the *groESL* operon (Lecointe et al, 2004; Meima et al, 2001).

The genetic constructs of the cassettes for the production of catechol were as follow:

20 *aroZ* of *Bacillus thuringiensis* (*Bt_aroZ*; SEQ ID NO: 3) or *quiC* of *Acinetobacter sp. ADP1* (*Ac_quiC*; SEQ ID NO: 7) encoding DHS dehydratase (EC 4.2.1.118) was placed under the control of a strong constitutive promoter and was followed by *aroY* of *Klebsiella pneumonia* (*Kp_aroY*, SEQ ID NO: 15) comprising the genes encoding the three subunits of the protocatechuate decarboxylase (EC 4.1.1.63) also controlled by a
25 strong constitutive promoter (cf. Figures 1 and 3).

To make seed cultures, individual colonies were picked to inoculate 25 ml of CMG2% medium (Peptone 2 g/L ; Yeast Extract 5 g/L ; Glucose 55 mM (20 g/L) ; MOPS acid 40 mM ; NH₄Cl 20 mM ; NaOH 10 mM ; KOH 10 mM ; CaCl₂.2H₂O 0,5 μM ;

Na₂SO₄.10H₂O 0.276 mM ; MgCl₂.6H₂O 0.528 mM ; (NH₄)₆(Mo₇)O₂₄.4H₂O 3 nM ;
 H₃BO₃ 0.4 μM ; CoCl₂.6H₂O 30 nM ; CuSO₄.5H₂O 10 nM ; MnCl₂ 0.25 μM ;
 ZnSO₄.7H₂O 10 nM ; D-Biotin 1 μg/L ; Niacin (nicotinic acid) 1 μg/L ; B6 vitamin 1
 μg/L ; B1 vitamin ; FeCl₃ 20 μM ; Sodium Citrate.2H₂O 20 μM ; K₂HPO₄ 5,7 mM)
 5 containing 1% glucose as the main carbon source, and cultured at 37°C and 250 rpm
 overnight.

Seed from log phase of growth was then inoculated into 1 ml of the mineral
 medium (0.4 g/L of yeast extract; (NH₄)₂SO₄ < 100 mM ; NaH₂PO₄.H₂O < 10 mM ;
 KCl < 10 mM ; Na₂SO₄ < 10 mM ; Acide citrique < 30 mM ; MgCl₂.6H₂O < 10 mM ;
 10 CaCl₂.2H₂O < 10 mM ; ZnCl₂ < 50 mg/L ; FeSO₄.7H₂O < 50 mg/L ; MnCl₂.4H₂O < 50
 mg/L ; CuSO₄ < 50 mg/L ; CoCl₂.6H₂O < 50 mg/L ; H₃BO₃ < 5 mg/L ; MES < 200 mM
 ; (NH₄)₆Mo₇O₂₄.4H₂O < 0,5 mM ; Glucose < 30 g/L (166 mM)) at an initial optical
 density at 600 nm (OD₆₀₀) of 0.4. The cultures were performed in deepwell at 48°C at
 250 rpm. At 72h of growth, aliquots of 500 μl of culture were taken and filtrated using
 15 0.22 μm membrane filter MF (Millipore). The filtrate was then injected in HPLC.

Results:

Recombinant *D. geothermalis* strains comprising the genetic construct illustrated
 in Figure 1, i.e. expressing *aroZ* of *Bacillus thuringiensis* and *aroY* of *Klebsiella*
pneumonia produced PCA and catechol (Figure 2). The same results were obtained with
 20 a recombinant *D. geothermalis* strain expressing *quiC* of *Acinetobacter* sp. *ADP1* and
aroY of *Klebsiella pneumonia*.

Exemple 2: Muconic acid production by recombinant *Deinococcus geothermalis*

Materiel & Methods:

25 *Deinococcus geothermalis* strains were genetically engineered to produce
 muconic acid.

Genomic DNAs were prepared using Dneasy&Blood QIAGEN Kit as indicated
 by the Manufacturer. Each gene was amplified and assembled by overlapping PCR.
 Insertion of DNA fragments into the chromosome of *D. geothermalis* was performed

using homologous recombination mechanism. Insertion cassettes comprised a nucleic acid sequence to be inserted into the chromosome flanked by 1500 bp region homologous to the sequence upstream or downstream the chromosomal target *aroE* gene.

For the expression of heterologous genes, strong constitutive promoters were used such as PtufA and PtufB promoters from the translation elongation factors Tu genes *tufA* (DR0309) and *tufB* (DR2050), or the promoter region PgroESL of the *groESL* operon (Lecointe et al, 2004; Meima et al, 2001).

The genetic constructs of the cassettes for the production of muconic acid were as follow:

10 *quiC* of *Acinetobacter* sp. ADP1 (*Ac_quiC*; SEQ ID NO: 7) or *aroZ* of *Bacillus thuringiensis* (*Bt_aroZ*; SEQ ID NO: 3) both encoding DHS dehydratase (EC 4.2.1.118) was placed under the control of strong constitutive promoter and was followed by *aroY* of *Klebsiella pneumonia* (SEQ ID NO: 15) encoding protocatechuate decarboxylase (EC 4.1.1.63) controlled by a strong constitutive promoter (Figures 1 and 3).

15 Each recombinant comprising an expression cassette as illustrated in Figure 1 or 3, was further modified by inserting an expression cassette comprising *catA* encoding catechol 1,2-dioxygenase from *Acinetobacter calcoaceticus* (*Ac.c_CatA*, SEQ ID NO: 24) under the control of strong constitutive promoter, in an IS sequence (IS66).

To make seed cultures, individual colonies were picked to inoculate 25 ml of 20 CMG2% medium (Peptone 2 g/L ; Yeast Extract 5 g/L ; Glucose 55 mM (20 g/L) ; MOPS acid 40 mM ; NH₄Cl 20 mM ; NaOH 10 mM ; KOH 10 mM ; CaCl₂.2H₂O 0,5 μM ; Na₂SO₄.10H₂O 0.276 mM ; MgCl₂.6H₂O 0.528 mM ; (NH₄)₆(Mo₇)O₂₄.4H₂O 3 nM ; H₃BO₃ 0.4 μM ; CoCl₂.6H₂O 30 nM ; CuSO₄.5H₂O 10 nM ; MnCl₂ 0.25 μM ; ZnSO₄.7H₂O 10 nM ; D-Biotin 1 μg/L ; Niacin (nicotinic acid) 1 μg/L ; B6 vitamin 1 25 μg/L ; B1 vitamin ; FeCl₃ 20 μM ; Sodium Citrate.2H₂O 20 μM ; K₂HPO₄ 5,7 mM) containing 1% glucose as the main carbon source, and cultured at 37°C and 250 rpm overnight.

Seed from log phase of growth was then inoculated into 1 ml of the mineral medium (as described above) at an initial optical density at 600 nm (OD₆₀₀) of 0.4. The 30 cultures were performed in deepwell either at 37°C or 48°C at 250 rpm. At 72h of growth,

aliquots of 500 µl of culture were taken and filtrated using 0.22 µm membrane filter MF (Millipore). The filtrate was then injected in HPLC.

Results:

Recombinant *D. geothermalis* strains comprising an expression cassette 5 comprising (a) *quiC* of *Acinetobacter sp. ADP1* and *aroY* of *Klebsiella pneumonia*, or (b) *aroZ* of *Bacillus thuringiensis* and *aroY* of *Klebsiella pneumonia*, and an expression cassette comprising *catA* of *Acinetobacter calcoaceticus* produced muconic acid (Figure 4 and 5).

Exemple 3: Muconic acid production by recombinant *Deinococcus* 10 *geothermalis*

Materiel & Methods:

Deinococcus geothermalis strains were genetically engineered to produce muconic acid.

Genomic DNAs were prepared using Dneasy&Blood QIAGEN Kit as indicated 15 by the Manufacturer. Each gene was amplified and assembled by overlapping PCR. Insertion of DNA fragments into the chromosome of *D. geothermalis* was performed using homologous recombination mechanism. Insertion cassettes comprised a nucleic acid sequence to be inserted into the chromosome flanked by 1500 bp region homologous to the sequence upstream or downstream the chromosomal target *aroE* gene.

20 For the expression of heterologous genes, strong constitutive promoters were used such as *PtufA* and *PtufB* promoters from the translation elongation factors Tu genes *tufA* (DR0309) and *tufB* (DR2050), or the promoter region *PgroESL* of the *groESL* operon (Lecointe et al, 2004; Meima et al, 2001).

The genetic constructs of the cassettes for the production of muconic acid were as 25 follow:

quiC of *Acinetobacter sp. ADP1* (*Ac_quiC*; SEQ ID NO: 7) or *aroZ* of *Bacillus thuringiensis* (*Bt_aroZ*; SEQ ID NO: 3) both encoding DHS dehydratase (EC 4.2.1.118) was placed under the control of strong constitutive promoter and was followed by *aroY*

of *Klebsiella pneumonia* (SEQ ID NO: 15) encoding protocatechuate decarboxylase (EC 4.1.1.63) controlled by a strong constitutive promoter (Figures 1 and 3).

Each recombinant comprising an expression cassette as illustrated in Figure 1 or 3, was further modified by inserting an expression cassette comprising *catA* encoding catechol 1,2-dioxygenase from *Candida albicans* (SEQ ID NO: 29) or *Bulkholderia xenovorans* (SEQ ID NO: 31), under the control of strong constitutive promoter, in an IS sequence (IS66).

To make seed cultures, individual colonies were picked to inoculate 25 ml of CMG2% medium (Peptone 2 g/L ; Yeast Extract 5 g/L ; Glucose 55 mM (20 g/L) ; MOPS acid 40 mM ; NH₄Cl 20 mM ; NaOH 10 mM ; KOH 10 mM ; CaCl₂.2H₂O 0,5 μM ; Na₂SO₄.10H₂O 0.276 mM ; MgCl₂.6H₂O 0.528 mM ; (NH₄)₆(Mo₇)O₂₄.4H₂O 3 nM ; H₃BO₃ 0.4 μM ; CoCl₂.6H₂O 30 nM ; CuSO₄.5H₂O 10 nM ; MnCl₂ 0.25 μM ; ZnSO₄.7H₂O 10 nM ; D-Biotin 1 μg/L ; Niacin (nicotinic acid) 1 μg/L ; B6 vitamin 1 μg/L ; B1 vitamin ; FeCl₃ 20 μM ; Sodium Citrate.2H₂O 20 μM ; K₂HPO₄ 5,7 mM) containing 1% glucose as the main carbon source, and cultured at 37°C and 250 rpm overnight.

Seed from log phase of growth was then inoculated into 1 ml of the mineral medium (as described above) at an initial optical density at 600 nm (OD₆₀₀) of 0.4. The cultures were performed in deepwell either at 48°C pH6 at 250 rpm. At 72h of growth, aliquots of 500 μl of culture were taken and filtrated using 0.22 μm membrane filter MF (Millipore). The filtrate was then injected in HPLC.

Results:

Recombinant *D. geothermalis* strains comprising an expression cassette comprising (a) *quiC* of *Acinetobacter sp. ADP1* and *aroY* of *Klebsiella pneumonia*, or (b) *aroZ* of *Bacillus thuringiensis* and *aroY* of *Klebsiella pneumonia*, and an expression

cassette comprising *catA* of *Candida albicans* or *Bulkholderia xenovorans*, produced muconic acid.

Example 4: Catechol production by recombinant *Deinococcus geothermalis* cultivated in fermenter

5 A *Deinococcus geothermalis* strain was genetically engineered to produce catechol as described in example 1 and figure 1.

Genomic DNAs were prepared using Dneasy&Blood QIAGEN Kit as indicated by the Manufacturer. Each gene was amplified and assembled by overlapping PCR. Insertion of DNA fragments into the chromosome of *D. geothermalis* was performed
10 using homologous recombination mechanism. Insertion cassettes comprised a nucleic acid sequence to be inserted into the chromosome flanked by 1500 bp region homologous to the sequence upstream or downstream the chromosomal target *aroE* gene.

For the expression of heterologous genes, strong constitutive promoters were used such as PtufA and PtufB promoters from the translation elongation factors Tu genes *tufA*
15 (DR0309) and *tufB* (DR2050), or the promoter region PgroESL of the *groESL* operon (Lecointe et al, 2004; Meima et al, 2001).

The genetic construct of the cassette for the production of catechol was as follow:

aroZ of *Bacillus thuringiensis* (*Bt_aroZ*; SEQ ID NO: 3) encoding DHS dehydratase (EC 4.2.1.118) was placed under the control of a strong constitutive promoter
20 and was followed by *aroY* of *Klebsiella pneumonia* (*Kp_aroY*, SEQ ID NO: 15) comprising the genes encoding the three subunits of the protocatechuate decarboxylase (EC 4.1.1.63) also controlled by a strong constitutive promoter (cf. Figures 1 and 3).

To make seed cultures, individual colonies were picked to inoculate 25 ml of CMG2% medium (Peptone 2 g/L ; Yeast Extract 5 g/L ; Glucose 55 mM (20 g/L) ; MOPS
25 acid 40 mM ; NH₄Cl 20 mM ; NaOH 10 mM ; KOH 10 mM ; CaCl₂.2H₂O 0,5 μM ; Na₂SO₄.10H₂O 0.276 mM ; MgCl₂.6H₂O 0.528 mM ; (NH₄)₆(Mo₇)O₂₄.4H₂O 3 nM ; H₃BO₃ 0.4 μM ; CoCl₂.6H₂O 30 nM ; CuSO₄.5H₂O 10 nM ; MnCl₂ 0.25 μM ; ZnSO₄.7H₂O 10 nM ; D-Biotin 1 μg/L ; Niacin (nicotinic acid) 1 μg/L ; B6 vitamin 1

$\mu\text{g/L}$; B1 vitamin ; FeCl_3 20 μM ; Sodium Citrate.2H₂O 20 μM ; K_2HPO_4 5,7 mM) containing 1% glucose as the main carbon source, and cultured at 37°C and 250 rpm overnight.

Seed from log phase of growth was then inoculated into 1 L of the mineral medium
 5 pH 6 (0.4 g/L of yeast extract; $(\text{NH}_4)_2\text{SO}_4$ < 100 mM ; $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ < 10 mM ; KCl < 10 mM ; Na_2SO_4 < 10 mM ; Acide citrique < 30 mM ; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ < 10 mM ; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ < 10 mM ; ZnCl_2 < 50 mg/L ; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ < 50 mg/L ; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ < 50 mg/L ; CuSO_4 < 50 mg/L ; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ < 50 mg/L ; H_3BO_3 < 5 mg/L ; MES < 200 mM ; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ < 0,5 mM ; Glucose < 30 g/L (166 mM)) at an initial optical
 10 density at 600 nm (OD₆₀₀) of 0.4. The cultures were performed in reactor (fermenter) at 48°C. At 64h of growth, aliquots of 500 μl of culture were taken and filtrated using 0.22 μm membrane filter MF (Millipore). The filtrate was then injected in HPLC.

Results

The recombinant *Deinococcus* strain was able to produce up to 1000 mg/L of catechol in
 15 fermenter at 64 hours of cultivation.

Example 5: cis,cis muconic acid production by recombinant *Deinococcus geothermalis* cultivated in fermenter

Deinococcus geothermalis strains were genetically engineered to produce muconic acid as described in example 2 and figure 1.

20 Genomic DNAs were prepared using Dneasy&Blood QIAGEN Kit as indicated by the Manufacturer. Each gene was amplified and assembled by overlapping PCR. Insertion of DNA fragments into the chromosome of *D. geothermalis* was performed using homologous recombination mechanism. Insertion cassettes comprised a nucleic acid sequence to be inserted into the chromosome flanked by 1500 bp region homologous
 25 to the sequence upstream or downstream the chromosomal target *aroE* gene.

For the expression of heterologous genes, strong constitutive promoters were used such as PtufA and PtufB promoters from the translation elongation factors Tu genes *tufA* (DR0309) and *tufB* (DR2050), or the promoter region PgroESL of the *groESL* operon (Lecointe et al, 2004; Meima et al, 2001).

The genetic constructs of the cassettes for the production of muconic acid were as follow:

aroZ of *Bacillus thuringiensis* (*Bt_aroZ*; SEQ ID NO: 3) encoding DHS dehydratase (EC 4.2.1.118) was placed under the control of strong constitutive promoter 5 and was followed by *aroY* of *Klebsiella pneumonia* (SEQ ID NO: 15) encoding protocatechuate decarboxylase (EC 4.1.1.63) controlled by a strong constitutive promoter (Figures 1 and 3).

Recombinant comprising an expression cassette as illustrated in Figure 1, was further modified by inserting an expression cassette comprising *catA* encoding catechol 10 1,2-dioxygenase from *Acinetobacter calcoaceticus* (*Ac.c_CatA*, SEQ ID NO: 24) under the control of strong constitutive promoter, in an IS sequence (IS66).

To make seed cultures, individual colonies were picked to inoculate 25 ml of CMG2% medium (Peptone 2 g/L ; Yeast Extract 5 g/L ; Glucose 55 mM (20 g/L) ; MOPS acid 40 mM ; NH₄Cl 20 mM ; NaOH 10 mM ; KOH 10 mM ; CaCl₂.2H₂O 0,5 μM ; 15 Na₂SO₄.10H₂O 0.276 mM ; MgCl₂.6H₂O 0.528 mM ; (NH₄)₆(Mo₇)O₂₄.4H₂O 3 nM ; H₃BO₃ 0.4 μM ; CoCl₂.6H₂O 30 nM ; CuSO₄.5H₂O 10 nM ; MnCl₂ 0.25 μM ; ZnSO₄.7H₂O 10 nM ; D-Biotin 1 μg/L ; Niacin (nicotinic acid) 1 μg/L ; B6 vitamin 1 μg/L ; B1 vitamin ; FeCl₃ 20 μM ; Sodium Citrate.2H₂O 20 μM ; K₂HPO₄ 5,7 mM) containing 1% glucose as the main carbon source, and cultured at 37°C and 250 rpm 20 overnight.

Seed from log phase of growth was then inoculated into 50 ml of the mineral medium (as described above) at an initial optical density at 600 nm (OD₆₀₀) of 0.4. The cultures was performed in 500ml-flask at pH6, 37°C and at 250 rpm. At 144h of growth, aliquots of 500 μl of culture were taken and filtrated using 0.22 μm membrane filter MF 25 (Millipore). The filtrate was then injected in HPLC.

Results

The recombinant *Deinococcus* strain was able to produce up to 175 mg/L of muconic acid in flask at 144 hours of cultivation.

REFERENCES

- Ben-Aroya et al. *Methods Enzymol.* 2010; 470: 181–204.
- Bentley. *Crit Rev Biochem Mol Biol.* 1990;25(5):307-84
- Cao, B.; Geng, A.; Loh, K.C.; *Appl. Microbiol. Biotechnol.* 81, 99-107 (2008)
- 5 Caposio et al. *Res Microbiol.* 2002 Mar;153(2):69-74.
- Curran et al. *Metab Eng.* 2013 Jan;15:55-66.
- Draths et al. (1992) *Journal of the American Chemical Society* 114, 3956- 3962.
- Draths, K.; Frost, J.; *J. Am. Chem. Soc.* 117, 2395-2400 (1995)
- Elsemore et al. *Journal of Bacteriology* (1995), 177 (20), 5971-8
- 10 Fox et al., *Biochemistry.* 2008 Nov 25;47(47):12251-3
- Grant DJ, Patel JC. *Antonie Leeuwenhoek* 35, 325-343 (1969)
- Han et al. *Sci Rep.* 2015 Aug 26;5:13435.
- Hansen et al. *Appl Environ Microbiol.* 2009 May;75(9):2765-74
- He, Z.; Wiegel, J.; *J. Bacteriol.* 178, 3539-3543 (1996)
- 15 Hu et al. (2003) *J Basic Microbiol* 43, 399-406.
- Jimenez et al. *Environmental Microbiology* (2002), 4 (12), 824-841
- Lamb et al. *Biochem. J.* 284, 181-187
- Lecointe, et al. 2004. *Mol Microbiol* 53: 1721–1730
- Lutke-Eversloh, T., and Stephanopoulos, G. (2007) *Appl Microbiol Biotechnol*
 20 75, 103-110.
- Makarova et al. 2001, *Microbiol Mol Biol Rev* 65: 44–79
- Meima et al. 2001. *J Bacteriol* 183: 3169–3175
- Molina et al. *Genome Announc.* 2013 Jul 18;1(4). pii: e00496-13.
- Mottern, J. *Am. Chem. Soc.*, 1934, 56 (10), pp 2107–2108
- 25 Musser, M. T. 2005. Adipic acid. In : Wiley-VCH (Ed.), *ULLMANN'S Encyclopedia of Industrial Chemistry*. Wiley-VCH, Weinheim
- Neidle, E.L.; Ornston, L.N.; *J. Bacteriol.* 168, 815-820 (1986)

- Niu et al., 2002. Benzene-free synthesis of adipic acid. *Biotechnol. Prog.* 18, 201–211.
- Rutledge et al. *Gene*. 1984 Dec;32(3):275-87.
- Shinagawa et al. *Biosci Biotechnol Biochem.* 2010;74(5):1084-8
- 5 Shumilin et al (1999) *Structure* 7, 865-875
- Tsai, S.C.; Li, Y.K.; *Arch. Microbiol.* 187, 199-206 (2007)
- Weber et al. 2012. *Appl. Environ. Microbiol.* 78, 8421–8430.
- Yoshida et al. *Biotechnol. Lett.* 32, 701-705 (2010)

CLAIMS

1. A recombinant *Deinococcus* bacterium expressing a heterologous polypeptide exhibiting 3-dehydroshikimate dehydratase activity and a heterologous polypeptide exhibiting protocatechuate decarboxylase activity.

5 2. The recombinant bacterium of claim 1, wherein said bacterium further expresses a heterologous polypeptide exhibiting catechol 1,2-dioxygenase activity.

3. The recombinant bacterium according to claim 1 or 2, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratases from *Bacillus thuringiensis*, *Podospora anserina*, ,
10 *Klebsiella pneumoniae*, *Acinetobacter calcoaceticus*, *Acinetobacter sp. ADP1*, *Acinetobacter baylyi*, *Neurospora crassa*, *Aspergillus nidulans*, *Gluconobacter oxydans* and *Pseudomonas putida*, in particular *Pseudomonas putida* KT2440 and *Pseudomonas putida* H8234.

4. The recombinant bacterium according to any of claims 1 to 3, wherein said
15 polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratases from *Bacillus thuringiensis*, *Podospora anserina*, *Pseudomonas putida* and *Acinetobacter sp. ADP1*, preferably from *Bacillus thuringiensis*, *Podospora anserina* and *Acinetobacter sp. ADP1*, and more preferably from *Bacillus thuringiensis* and *Acinetobacter sp. ADP1*.

20 5. The recombinant bacterium according to any of claims 1 to 4, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratases of SEQ ID NO: 4, 2, 6 and 8, and polypeptides exhibiting 3-dehydroshikimate dehydratase activity and having at least 60 % identity to SEQ ID NO: 4, 2, 6 or 8.

25 6. The recombinant bacterium according to any of claims 1 to 5, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratases of SEQ ID NO: 4 and 8, and polypeptides exhibiting 3-dehydroshikimate dehydratase activity and having at least 60 % identity to SEQ ID NO: 4 or 8.

7. The recombinant bacterium according to any of claims 1 to 6, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratase of SEQ ID NO: 4, and polypeptides exhibiting 3-dehydroshikimate dehydratase activity and having at least 60 % identity to
5 SEQ ID NO: 4.

8. The recombinant bacterium according to any of claims 1 to 6, wherein said polypeptide exhibiting 3-dehydroshikimate dehydratase activity is selected from the group consisting of 3-dehydroshikimate dehydratase of SEQ ID NO: 8, and polypeptides exhibiting 3-dehydroshikimate dehydratase activity and having at least 60 % identity to
10 SEQ ID NO: 8.

9. The recombinant bacterium according to any of claims 1 to 8, wherein said polypeptide exhibiting protocatechuate decarboxylase activity is selected from the group consisting of 3-protocatechuate decarboxylases from *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Sedimentibacter hydroxybenzoicus*, preferably from *Klebsiella*
15 *pneumoniae*.

10. The recombinant bacterium according to any of claims 1 to 9, wherein said polypeptide exhibiting protocatechuate decarboxylase activity is selected from the group consisting of (i) a PCA decarboxylase comprising polypeptides of SEQ ID NO: 10, 12 and 14, (ii) a PCA decarboxylase comprising polypeptides of SEQ ID NO: 17, 19 and 21,
20 (iii) the PCA decarboxylase of SEQ ID NO: 23, and (iv) PCA decarboxylases having at least 60 % identity to SEQ ID NO: 10, 12, 14, 17, 19, 21 or 23.

11. The recombinant bacterium according to any of claims 1 to 10, wherein said polypeptide exhibiting protocatechuate decarboxylase activity is selected from the group consisting of a PCA decarboxylase comprising polypeptides of SEQ ID NO: 10, 12 and
25 14, and PCA decarboxylases having at least 60 % identity to SEQ ID NO: 10, 12 or 14.

12. The recombinant bacterium according to any of claims 2 to 11, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenases from *Acinetobacter radioresistens*, *Acinetobacter calcoaceticus*, *Candida albicans*, *Bulkholderia mallei*, *Bulkholderia*
30 *xenovorans*, *Pseudomonas putida*, *Stenotrophomonas maltophilia* KB2, *Cupriavidus*

metallidurans CH34, *Burkholderia* sp. TH2, *Rhodococcus opacus*, *Rhodococcus erythropolis* and *Acinetobacter* sp. ADP1.

13. The recombinant bacterium according to any of claims 2 to 12, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenases from *Acinetobacter radioresistens*, *Acinetobacter calcoaceticus*, *Candida albicans*, *Burkholderia mallei*, *Burkholderia xenovorans* and *Pseudomonas putida*, preferably from *Acinetobacter calcoaceticus*, *Candida albicans* and *Burkholderia xenovorans*.

14. The recombinant bacterium according to any of claims 2 to 12, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenases of SEQ ID NO: 25, 27, 29, 31, 33, 34, 35, 36, 37, 38, 39, 40 and 41 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 25, 27, 29, 31, 33, 34, 35, 36, 37, 38, 39, 40 or 41.

15. The recombinant bacterium according to any of claims 2 to 14, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenases of SEQ ID NO: 25, 29 and 31 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 25, 29 or 31.

16. The recombinant bacterium according to any of claims 2 to 15, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 25 and polypeptides exhibiting catechol 1,2-dioxygenase activity and having at least 60 % identity to SEQ ID NO: 25.

17. The recombinant bacterium according to any of claims 2 to 14, wherein said polypeptide exhibiting catechol 1,2-dioxygenase activity is selected from the group consisting of catechol 1,2-dioxygenase of SEQ ID NO: 41 and a variant thereof comprising at least one substitution at position corresponding to residue G72, L73 or P76, preferably at least one substitution or combination of substitutions selected from G72A, L73F, P76A, G72A+L73F, G72A+P76A, P76A+L73F, G72A+L73F+P76A, more preferably selected from G72A, P76A, L73F and L73F+P76A.

18. The recombinant bacterium according to any of claims 1 to 17, wherein the endogenous biosynthetic pathway converting 3-dehydroshikimate to chorismate is blocked or reduced.

19. The recombinant bacterium according to claim 18, wherein the endogenous
5 gene encoding shikimate dehydrogenase (AroE) is inactivated.

20. The recombinant bacterium according to claim 18 or 19, wherein the endogenous shikimate dehydrogenase activity is reduced.

21. The recombinant bacterium according to any of claims 1 to 20, wherein the endogenous biosynthetic pathway converting protocatechuate to oxoadipate is blocked or
10 reduced.

22. The recombinant bacterium according to claim 21, wherein the endogenous biosynthetic pathway converting protocatechuate to oxoadipate is blocked by inactivation one or several endogenous genes selected from genes encoding protocatechuate 3,4-dioxygenase, 3-carboxy-cis,cis-muconate cycloisomerase and 3-oxoadipate enol-
15 lactonase.

23. The recombinant bacterium according to any of claims 1 to 22, wherein one or several of the enzymes involved in the conversion of phosphoenolpyruvate and erythrose 4-phosphate to 3-dihydroshikimate, preferably selected from AroF, AroG, AroH, AroB and AroD, are overexpressed and/or are feedback inhibition resistant
20 enzymes.

24. The recombinant bacterium according to claim 23, wherein the recombinant bacterium expresses a feedback inhibition resistant DAHP synthase.

25. The recombinant bacterium according to claim 24, wherein the feedback inhibition resistant DAHP synthase is a variant of the *Deinococcus* DAHP synthase set
25 forth in SEQ ID NO: 46 and comprises at least one substitution at position corresponding to residue N13, P156 or S186 of SEQ ID NO: 46, preferably selected from N13K, P156L, S186F, N13K+ P156L, N13K+S186F, P156L+S186F and N13K+ P156L+S186F.

26. The recombinant bacterium of any of claims 1 to 25, further expresses a heterologous polypeptide exhibiting catechol-O-methyltransferase activity.

27. The recombinant bacterium of claim 26, wherein the polypeptide exhibiting catechol-O-methyltransferase activity is selected from the group consisting of COMT from *Mycobacterium vanbaalenii* (SEQ ID NO: 54) and any polypeptide exhibiting COMT activity and having at least 60 % identity to SEQ ID NO: 54.

5 28. A method of producing *cis-cis* muconic acid comprising culturing a recombinant *Deinococcus* bacterium according to any of claims 2 to 25 under conditions suitable to produce *cis-cis* muconic acid, and optionally recovering said *cis-cis* muconic acid.

29. A method of producing catechol comprising culturing a recombinant
10 *Deinococcus* bacterium according to any of claims 1 to 25 under conditions suitable to produce catechol, and optionally recovering said catechol.

30. A method of producing *cis-cis* muconic acid comprising (i) producing catechol according to the method of claim 29, (ii) enzymatically converting catechol to *cis-cis* muconic acid, and optionally (iii) recovering said *cis-cis* muconic acid.

15 31. The method according to any of claims 28 to 30, wherein the culture of the recombinant *Deinococcus* bacterium under conditions suitable to produce *cis-cis* muconic acid or catechol is performed at a temperature comprised between 37 and 55°C.

32. A method of producing adipic acid comprising producing *cis-cis* muconic acid according to the method of claim 28, 30 or 31 and reducing said *cis-cis* muconic acid to
20 produce adipic acid, and optionally recovering said adipic acid.

33. A method of producing *cis-trans* and/or *trans-trans* muconic acid comprising producing *cis-cis* muconic acid according to the method of claim 28, 30 or 31 and isomerizing said *cis-cis* muconic acid to produce *cis-trans* and/or *trans-trans* muconic acid, and optionally recovering said *cis-trans* and/or *trans-trans* muconic acid.

25 34. A method of producing gaiacol comprising (i) culturing a recombinant *Deinococcus* bacterium according to claim 26 or 27 under conditions suitable to produce gaiacol, and optionally (ii) recovering said gaiacol.

35. A method of producing vanillin comprising (i) culturing a recombinant *Deinococcus* bacterium according to claim 26 or 27 under conditions suitable to produce gaiacol, (ii) converting gaiacol to vanillin and, optionally (iii) recovering said vanillin.

36. The method of claim 35, further comprising recovering gaiacol produced in step (i) before conversion.

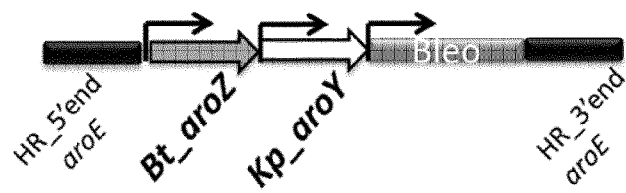


Figure 1

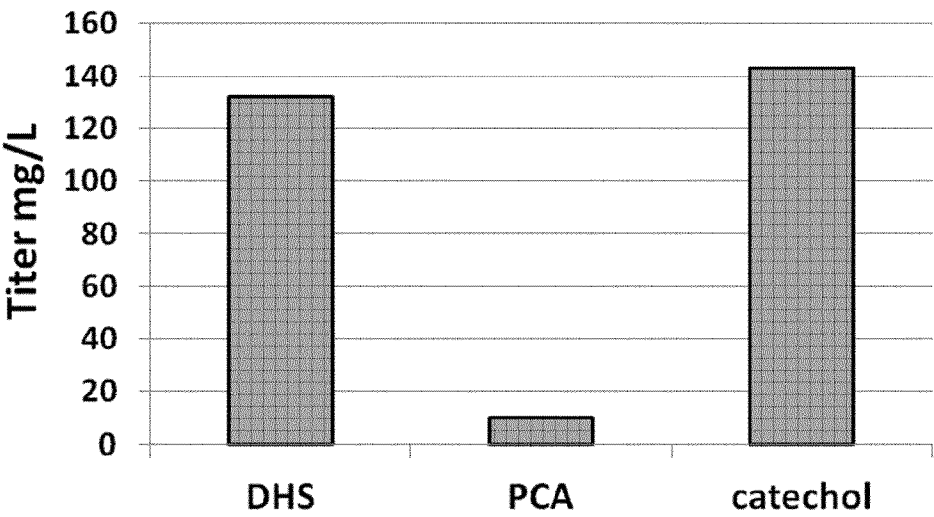


Figure 2

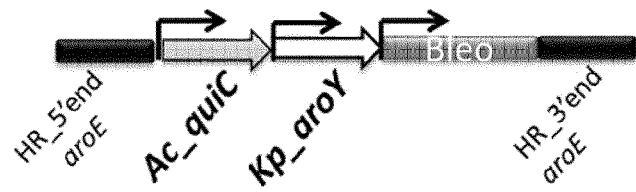


Figure 3

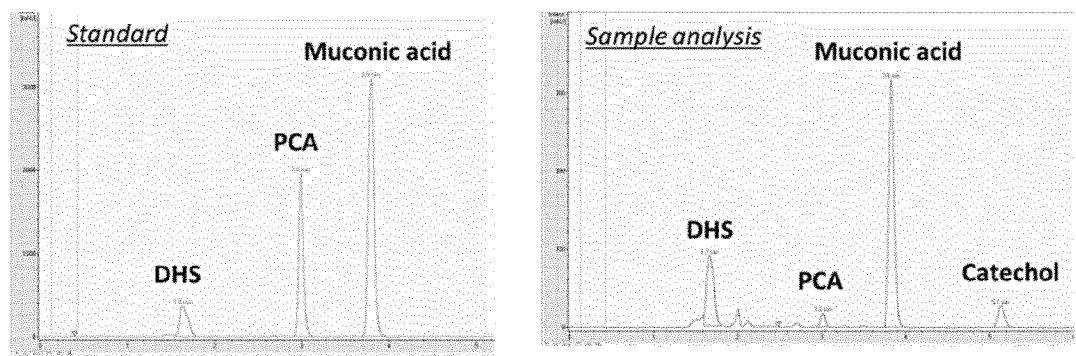


Figure 4

3/3

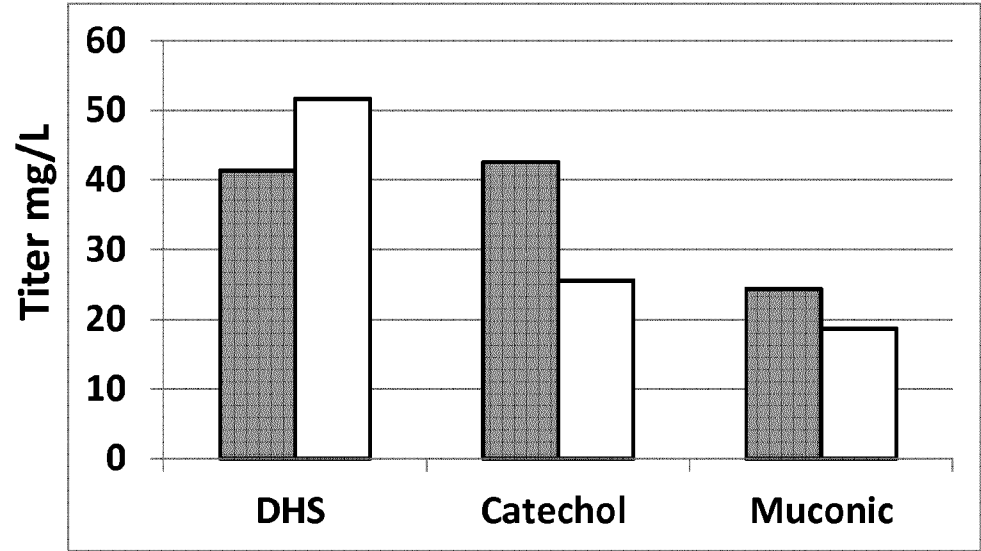


Figure 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2016/064752

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/064752

A. CLASSIFICATION OF SUBJECT MATTER INV. C12N9/08 C12N9/02 C12N9/88 C12P7/22 C12P7/46 C12R1/01 ADD. According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) C12N C12P Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, Sequence Search, BIOSIS, EMBASE, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2009/113853 A2 (DSM IP ASSETS BV [NL]; WU LIANG [NL]; TREFZER AXEL CHRISTOPH [NL]; DE) 17 September 2009 (2009-09-17) the whole document -----	1-36
Y	NIU WEI ET AL: "Benzene-free synthesis of adipic acid", BIOTECHNOLOGY PROGRESS, AMERICAN INSTITUTE OF CHEMICAL ENGINEERS, US, vol. 18, no. 2, 1 March 2002 (2002-03-01), pages 201-211, XP002568326, ISSN: 8756-7938, DOI: 10.1021/BP010179X [retrieved on 2002-02-12] cited in the application the whole document ----- -/-	1-36
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 30 August 2016		Date of mailing of the international search report 09/09/2016
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Oderwald, Harald

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/064752

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2013/116244 A1 (MYRIANT CORP [US]) 8 August 2013 (2013-08-08) the whole document	23
A	WO 2011/085311 A1 (DRATHS CORP [US]; BUI VU [US]; LAU MAN KIT [US]; MACRAE DOUG [US]) 14 July 2011 (2011-07-14) the whole document	28-33
A	WO 2012/106257 A1 (LOS ALAMOS NAT SECURITY LLC [US]; KOPPISCH ANDREW T [US]; FOX DAVID T) 9 August 2012 (2012-08-09) the whole document	1
A	WO 2010/130812 A1 (DEINOVE [FR]; CENTRE NAT RECH SCIENT [FR]; UNIV MONTPELLIER I [FR]; BI) 18 November 2010 (2010-11-18) cited in the application the whole document	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2016/064752

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009113853	A2	17-09-2009	
		AU 2009224087 A1	17-09-2009
		CN 102027125 A	20-04-2011
		EA 201001444 A1	29-04-2011
		EP 2252698 A2	24-11-2010
		JP 2011512868 A	28-04-2011
		TW 201002824 A	16-01-2010
		UA 106040 C2	25-07-2014
		US 2011091944 A1	21-04-2011
		US 2014178949 A1	26-06-2014
		WO 2009113853 A2	17-09-2009
WO 2013116244	A1	08-08-2013	
		CA 2862051 A1	08-08-2013
		CN 104220587 A	17-12-2014
		EP 2809771 A1	10-12-2014
		JP 2015504688 A	16-02-2015
		KR 20140131939 A	14-11-2014
		US 2015044755 A1	12-02-2015
		WO 2013116244 A1	08-08-2013
WO 2011085311	A1	14-07-2011	
		BR 112012016855 A2	15-09-2015
		CA 2786405 A1	14-07-2011
		CN 102985537 A	20-03-2013
		DK 2521770 T3	29-02-2016
		EP 2521770 A1	14-11-2012
		JP 2013516196 A	13-05-2013
		JP 2015142593 A	06-08-2015
		US 2013030215 A1	31-01-2013
		WO 2011085311 A1	14-07-2011
WO 2012106257	A1	09-08-2012	
		US 2012196339 A1	02-08-2012
		US 2013252294 A1	26-09-2013
		WO 2012106257 A1	09-08-2012
WO 2010130812	A1	18-11-2010	
		AU 2010247336 A1	19-01-2012
		CA 2761132 A1	18-11-2010
		CN 102482637 A	30-05-2012
		EA 201101626 A1	30-05-2012
		EP 2251414 A1	17-11-2010
		EP 2430149 A1	21-03-2012
		JP 5766182 B2	19-08-2015
		JP 2012526538 A	01-11-2012
		US 2012052540 A1	01-03-2012
		WO 2010130812 A1	18-11-2010
		ZA 201109163 B	30-01-2013