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(54) **Titre : PROCEDE DE PRODUCTION DE TERPENES OU DE TERPENOIDES**
(54) **Title: METHOD OF PRODUCING TERPENES OR TERPENOIDS**

(57) **Abrégé/Abstract:**

The present invention relates to arecombinant Deinococcus bacterium exhibiting enhanced 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate (MEP/DXP) pathway, and its use for producing terpene or terpenoid compounds.



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(54) Title: METHOD OF PRODUCING TERPENES OR TERPENOIDS

(57) Abstract: The present invention relates to arecombinant Deinococcus bacterium exhibiting enhanced 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate (MEP/DXP) pathway, and its use for producing terpene or terpenoid compounds.

Method of producing terpenes or terpenoids

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 isoprenoid compounds in genetically modified bacteria.

BACKGROUND OF THE INVENTION

Isoprenoids, also referred to as terpenes or terpenoids, are the most diverse class of natural products. Isoprenoids, which contain monoterpenes, sesquiterpenes, diterpenes and triterpenes as well as carotenoids, are the subject of numerous industrial applications, as they are found, for example, in pharmaceuticals, nutraceuticals, flavors, fragrances, cosmetics, colorants and agrichemicals.

Despite the great diversity in isoprenoid compounds, all are biosynthesized from a common C-5 precursor, isopentenyl pyrophosphate (IPP). This precursor can be synthesized via the mevalonate pathway (MEV) or via the non-mevalonate pathway (or 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate (MEP/DXP) pathway) leading to the formation of IPP and its isomer dimethylallyl pyrophosphate (DMAPP). DMAPP and IPP are then condensed to generate geranyl diphosphate (GPP) which is further converted with IPP into farnesyl diphosphate (FPP). FPP is further condensed with IPP to form geranylgeranyl diphosphate (GGPP). GPP, FPP and GGPP are the precursors of monoterpenes, sesquiterpenes, diterpenes, triterpenes and carotenoids.

Currently, isoprenoids are mainly extracted from plants or produced by chemical synthesis but suffer from low yields and high production costs.

Metabolic engineering of microorganisms offers an alternative and promising approach for the economical production of large amount of isoprenoids from cheap carbon sources. In this respect, a number of isoprenoids have been already produced by recombinant microorganism. *E. coli* and *S. cerevisiae* which can be genetically

manipulated very easily, have often been employed as host cells. For example, limonene was produced from a recombinant *E. coli* expressing the GPP synthase gene from grand fir and the (-)-limonene synthase gene from spearmint (Carter et al., 2003), or linalool production was obtained in a recombinant *S. cerevisiae* expressing the (S)-linalool
5 synthase gene from *Clarkia breweri* (Herrero et al., 2008).

Although engineering microorganisms to produce isoprenoids has shown great promise, it has been postulated that the total amount of isoprenoids is limited by the availability of isoprenoid precursors, i.e. IPP and DMAPP. Engineering an increased supply of isoprenoid precursors is thus necessary to increase the production. However, it
10 was observed that genetic manipulations of the complex MEP pathway can impact cellular growth rates and isoprene production, in particular due to accumulation of toxic metabolic intermediates such as IPP or DMAPP (e.g. Sivy et al., 2011) or metabolic burden on the host caused by depletion of pyruvate from the cells (Brown et al., 2010).

SUMMARY OF THE INVENTION

15 In a first aspect, the present invention provides an isoprenoid overproducing *Deinococcus* bacterium wherein the MEP pathway has been successfully engineered in order to increase the carbon flux to IPP and DMAPP.

The present invention thus relates to a recombinant *Deinococcus* bacterium exhibiting enhanced 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-
20 phosphate (MEP/DXP) pathway, in particular a bacterium wherein at least one enzymatic activity selected from the group consisting of DXP synthase (DXS), DXP reductoisomerase (DXR), IspD, IspE, IspF, IspG, IspH and IPP isomerase activities (IDI), is increased. Preferably, said at least one enzymatic activity is increased by overexpression of at least one gene selected from the group consisting of native,
25 homologous or heterologous *dxs*, *dxr*, *ispD*, *ispE*, *ispF*, *ispG*, *ispH* and *idi* genes.

Preferably, the recombinant bacterium of the invention is genetically modified to increase DXS activity and/or IDI activity. In particular, said recombinant bacterium may overexpress a native, homologous or heterologous *idi* gene and/or overexpress a native, homologous or heterologous *dxs* gene.

The bacterium of the invention may further exhibit an increased FPP synthase activity. Preferably, this increase is due to the overexpression of a native, homologous or heterologous *ispA* gene.

Preferably, the *ipsA* gene encodes a polypeptide selected from the group
5 consisting of

- a) a polypeptide comprising all or an active part of the amino acid sequence of SEQ ID NO: 47;
- b) a polypeptide having an amino acid sequence having at least 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% sequence identity to SEQ ID NO: 47;
- 10 c) a polypeptide encoded by a nucleotide sequence having at least 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% sequence identity to SEQ ID NO: 46; or
- d) a polypeptide which is encoded by a nucleic acid sequence which is capable of hybridizing under medium/high stringency, preferably high or very high, conditions with
15 or (i) the nucleic acid sequence set forth in SEQ ID NO: 46, (ii) its complementary strand, or (iii) a subsequence of (i) or (ii).

Preferably, the FPP synthase further exhibits dimethylallyltransferase activity and/or geranylgeranyl diphosphate synthase activity, more preferably dimethylallyltransferase activity and geranylgeranyl diphosphate synthase activity.

The bacterium of the invention may also further comprise a mutated DXP synthase
20 comprising an asparagine at position corresponding to position 222 of SEQ ID NO:52 and/or a cysteine at position corresponding to position 244 of SEQ ID NO:52, preferably a mutated DXP synthase comprising a cysteine at position corresponding to position 244 of SEQ ID NO: 52. In particular, the recombinant *Deinococcus* bacterium may express a gene encoding R244C mutant of the DXP synthase from *D. radiopugnans* (SEQ ID NO: 8), a gene encoding R238C mutant of the DXP synthase from *D. yunweiensis* (SEQ ID
25 NO: 14), and/or a gene encoding R241C mutant of the DXP synthase from *D. geothermalis* (SEQ ID NO: 56). Preferably, the recombinant *Deinococcus* bacterium expresses an improved (mutated) DXS enzyme and overexpresses a native, homologous or heterologous *idi* gene.

Preferably, the bacterium of the invention further comprises a gene encoding a heterologous terpene synthase.

The heterologous terpene synthase may be a monoterpene synthase preferably selected from the group consisting of geraniol synthase, 3S-linalool synthase, myrcene
5 synthase, R-linalool synthase, 1,8 cineol synthase, 4S-limonene synthase, R-limonene synthase, (-)- α -pinene synthase, (-)- β -pinene synthase, (-)-endo-fenchol synthase and (-)- α -terpineol synthase. More preferably, the heterologous terpene synthase is a geraniol synthase, a cineol synthase, a limonene synthase or a linalool synthase.

The heterologous terpene synthase may also be a sesquiterpene synthase
10 preferably selected from the group consisting (+)-epi-alpha-bisabolol synthase, germacrene A synthase, (E,E)-germacrene B synthase, germacrene C synthase, (-)-germacrene D synthase, valencene synthase, (3S, 6E)-nerolidol synthase, epi-cedrol synthase, patchoulol synthase, santalene synthase and δ -cadinene synthase. More preferably, the heterologous sesquiterpene synthase is a (+)-epi-alpha-bisabolol synthase.

15 The bacterium of the invention may further exhibit a reduced or suppressed lycopene beta-cyclase activity.

In a second aspect, the present invention relates to a recombinant *Deinococcus* bacterium wherein the FPP synthase activity is increased. Preferably, the FPP synthase activity is increased by overexpression of a native, homologous or heterologous *ipsA*
20 gene. Preferably, the *ipsA* gene encodes a polypeptide selected from the group consisting of

a) a polypeptide comprising all or an active part of the amino acid sequence of SEQ ID NO: 47;

b) a polypeptide having an amino acid sequence having at least 60, 65, 70, 75, 80,
25 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% sequence identity to SEQ ID NO: 47;

c) a polypeptide encoded by a nucleotide sequence having at least 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% sequence identity to SEQ ID NO: 46; or

d) a polypeptide which is encoded by a nucleic acid sequence which is capable of hybridizing under medium/high stringency, preferably high or very high, conditions with

(i) the nucleic acid sequence set forth in SEQ ID NO: 46, (ii) its complementary strand, or (iii) a subsequence of (i) or (ii).

Preferably, the FPP synthase further exhibits dimethylallyltransferase activity and/or geranylgeranyl diphosphate synthase activity, more preferably
5 dimethylallyltransferase activity and geranylgeranyl diphosphate synthase activity.

In a third aspect, the present invention relates to a recombinant *Deinococcus* bacterium comprising a mutated DXP synthase comprising an asparagine at position corresponding to position 222 of SEQ ID NO:52 and/or a cysteine at position corresponding to position 244 of SEQ ID NO:52, preferably a mutated DXP synthase
10 comprising a cysteine at position corresponding to position 244 of SEQ ID NO: 52. In particular, the recombinant *Deinococcus* bacterium may express a gene encoding R244C mutant of the DXP synthase from *D. radiopugnans* (SEQ ID NO: 8), a gene encoding R238C mutant of the DXP synthase from *D. yunweiensis* (SEQ ID NO: 14), and/or a gene encoding R241C mutant of the DXP synthase from *D. geothermalis* (SEQ ID NO: 56).

15 In a fourth aspect, the present invention relates to a recombinant *Deinococcus* bacterium expressing a gene encoding a heterologous terpene synthase, preferably a geraniol synthase or a (+)-epi-alpha-bisabolol synthase.

In a fifth aspect, the present invention relates to a recombinant *Deinococcus* bacterium wherein the endogenous lycopene beta-cyclase activity is reduced or
20 suppressed.

In a further aspect, the present invention relates to a use of a recombinant *Deinococcus* bacterium of the invention for producing an isoprenoid, preferably a monoterpene, a diterpene, a triterpene, a sesquiterpene or a carotenoid, more preferably a sesquiterpene or a carotenoid. In an embodiment, the isoprenoid is geraniol, geranic acid
25 and/or lycopene. In a particular embodiment, the isoprenoid is a carotenoid, preferably lycopene or another carotenoid compound derived from lycopene, in particular deinoxanthine. The present invention also relates to a method of producing an isoprenoid comprising (i) culturing a recombinant *Deinococcus* bacterium according to the invention under conditions suitable to produce the isoprenoid and optionally (ii) recovering the
30 isoprenoid. Preferably, the isoprenoid is a monoterpene, a diterpene, a triterpene, a sesquiterpene or a carotenoid, more preferably a sesquiterpene or a carotenoid. In an

embodiment, the isoprenoid is selected from the group consisting of geraniol, geranic acid and lycopene.

In another aspect, the present invention relates to a use of a recombinant *Deinococcus* bacterium of the invention for producing a geranyl ester. The present invention also relates to a method of producing a geranyl ester comprising (i) culturing a recombinant *Deinococcus* bacterium according to the invention under conditions suitable to produce geraniol and/or geranic acid, (ii) recovering geraniol and/or geranic acid, and (iii) converting geraniol and/or geranic acid into a geranyl ester, and optionally (iv) recovering the geranyl ester. Preferably, the geranyl ester is geranyl isobutyrate or *trans*-geranyl acetate.

In another aspect, the present invention relates to a use of a recombinant *Deinococcus* bacterium of the invention for producing a damascone, damascenone and/or ionone. The present invention also relates to a method of producing a damascone, damascenone and/or ionone comprising (i) culturing a recombinant *Deinococcus* bacterium according to the invention under conditions suitable to produce geraniol and/or geranic acid, (ii) recovering geraniol and/or geranic acid, and (iii) converting geraniol and/or geranic acid into the damascone, damascenone and/or ionone, and optionally (iv) recovering the damascone, damascenone and/or ionone.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: GCMS analysis of extraction at 24h of the culture comprising the recombinant *D. geothermalis* bacterium expressing the *Ocimum basilicum* GES.

Figure 2: GCMS analysis of extraction at 48h of the culture comprising the recombinant *D. geothermalis* bacterium expressing the *Ocimum basilicum* GES.

Figure 3: GCMS analysis of extraction at 72h of the culture comprising the recombinant *D. geothermalis* bacterium expressing the *Ocimum basilicum* GES.

Figure 4: Retention time of commercial geraniol.

Figure 5: Retention time of commercial geranic acid.

Figure 6: Difference of acetone extraction absorbance of *D. geothermalis* WT, the recombinant *D. geothermalis* bacterium wherein the *crtlm* gene was knockout, and lycopene standard.

Figure 7: Lycopene production of the recombinant *D. geothermalis* bacterium
5 wherein the *crtlm* gene was knockout with and without overexpression of the FPP synthase.

Figure 8: Geraniol production of the recombinant *D. geothermalis* bacterium expressing the GES of *Ocimum basilicum* with and without overexpression of the FPP synthase.

10 **Figure 9:** Geraniol production of recombinant *D. geothermalis* bacteria expressing the GES of *Ocimum basilicum* and overexpressing *D. yunweiensis* DXS, DXR, IspD, IspE, IspF, IspG, IspH or IDI.

Figure 10: Geraniol production of recombinant *D. geothermalis* bacteria expressing the GES of *Ocimum basilicum* and the wild-type DXS enzyme from *D.*
15 *yunweiensis* or the mutant R238C from *D. yunweiensis*.

Figure 11: Geraniol production of recombinant *D. geothermalis* bacteria expressing the GES of *Ocimum basilicum* and the wild-type DXS enzyme from *D. radiopugnans* or the mutant R244C.

Figure 12: Geraniol production of recombinant *D. geothermalis* bacteria
20 expressing the GES of *Ocimum basilicum* and the K170G mutant of the *D. geothermalis* FPPS.

Figure 13: Geraniol production of recombinant *D. geothermalis* bacteria expressing the GES of *Ocimum basilicum* and a mutated DXS and overexpressing DXR, IspD, IspE, IspF, IspG, IspH or IDI from *D. yunweiensis*.

25 **Figure 14:** Bisabolol production of the recombinant *D. geothermalis* bacterium expressing the (+)-epi-alpha-bisabolol synthase from *Streptomyces citricolor*. The wild-type bacterium does not produce bisabolol (data not shown).

Figure 15: Lycopene (carotenoid) production of the recombinant *D. geothermalis* bacterium wherein the *crtlm* gene was knockout with and without overexpression mutant DXS and IDI from *D. yunweiensis*.

Figure 16: Geraniol production of the recombinant *D. geothermalis* bacterium
5 expressing the GES of *Phyla dulcis* or *Cicer arietinum* with and without overexpression of the endogenous FPP synthase

Figure 17: Spectra of the deinoxanthin obtained by measuring the absorbance between 350 nm and 600 nm and normalizing said absorbances by the dry cell weight (DCW) for the wild-type bacterium and the bacterium overexpressing FPPS. The grey
10 spectrum corresponding to a deinoxanthin standard (purified deinoxanthin extracted from *D. radiodurans*).

Figure 18: Pictures of the cultures of the wild-type *D. geothermalis* bacterium and the *D. geothermalis* bacterium overexpressing endogenous FPPS.

Figure 19: Cineol production of the recombinant *D. geothermalis* bacterium
15 expressing the 1,8 cineole synthase gene from *Streptomyces clavuligerus*. Cineol is indicated by the arrow. The wild-type bacterium does not produce cineole (data not shown).

Figure 20: Limonene production of the recombinant *D. geothermalis* bacterium expressing the limonene synthase gene from *Mentha longifolia*. Limonene is indicated by
20 the arrow. The wild-type bacterium does not produce limonene (data not shown).

Figure 21: Linalool production (mg/L) of the recombinant *D. geothermalis* bacterium expressing the linalool synthase of *Perilla frutescens*. The wild-type bacterium does not produce linalool (data not shown).

DETAILED DESCRIPTION OF THE INVENTION

25 As indicated, the present invention relates to a *Deinococcus* bacterium genetically modified in order to exhibit improved production of isoprenoids.

In particular, the present application shows that the MEP pathway of *Deinococcus* bacteria can be engineered to increase the carbon flux to IPP and DMAPP. Indeed, the inventors identified several rate-limiting enzymes and showed that these enzymes can be

genetically engineered or overexpressed. They observed that isoprenoid production in these recombinant bacteria was significantly increased.

In addition, the inventors herein demonstrated that the production of isoprenoids in *Deinococcus* bacteria can be controlled to promote the production and/or accumulation of compounds of interest. In this respect, they identified and overexpressed the FPP synthase and showed that this overexpression promotes the production of isoprenoids having more than 10 C-atoms, such as diterpenes, triterpenes, sesquiterpenes, and carotenoids, to the detriment of monoterpenes. They also showed that the inactivation of the endogenous lycopene beta-cyclase induces an overproduction of lycopene.

Finally, the present application also demonstrates that *Deinococcus* bacteria, and in particular recombinant *Deinococcus* bacteria as described above, may be efficiently used to produce heterologous isoprenoid compounds via the expression of heterologous terpene synthase.

Definitions

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 limitation, *D. geothermalis*, *D. cellulolysiticus*, *D. radiodurans*, *D. proteolyticus*, *D. radiopugnans*, *D. radiophilus*, *D. grandis*, *D. indicus*, *D. frigens*, *D. saxicola*, *D. maricopenensis*, *D. marmoris*, *D. deserti*, *D. murrayi*, *D. aerius*, *D. aerolatus*, *D. aerophilus*, *D. aetherius*, *D. alpinitundrae*, *D. altitudinis*, *D. antarticus*, *D. apachensis*, *D. aquaticus*, *D. aquatilis*, *D. aquiradiocola*, *D. aquivivus*, *D. caeni*, *D. citri*, *D. claudionis*, *D. daejeonensis*, *D. depolymerans*, *D. enclensis*, *D. ficus*, *D. gobiensis*, *D. guangriensis*, *D. guilhemensis*, *D. hohokamensis*, *D. hopiensis*, *D. humi*, *D. misasensis*, *D. navajonensis*, *D. papagonensis*, *D. peraridilitoris*, *D. phoenicis*, *D. pimensis*, *D. piscis*, *D. puniceus*, *D. radiomollis*, *D. radioresistens*, *D. radiotolerans*, *D. reticulitermitis*, *D. roseus*, *D. sahariensis*, *D. soli*, *D. sonorensis*, *D. swuensis*, *D. wulumuqiensis*, *D. xibeiensis*, *D. xinjiangensis*, *D. yavapaiensis* or *D. yunweiensis* bacterium, or any combinations thereof. Preferably, the term “*Deinococcus*” refers to *D. geothermalis*, *D. radiopugnans*, *D. yunweiensi* and *D. murrayi*. In some embodiments, the term

“*Deinococcus*” refers to mesophile *Deinococcus* bacteria, preferably selected from *D. grandis*, *D. radiodurans*, *D. radiopugnans*, *D. yunweiensis* and *D. aquaticus*. In some other embodiments, the term “*Deinococcus*” refers to thermophile *Deinococcus* bacteria, preferably selected from *D. geothermalis*, *D. murrayi* and *D. maricopenis*. In preferred 5 embodiments, the term “*Deinococcus*” refers to *D. geothermalis*, *D. murrayi*, *D. maricopenis*, *D. grandis*, *D. radiodurans*, *D. radiopugnans*, *D. yunweiensis* and *D. aquaticus*. More preferably, the term “*Deinococcus*” refers to *D. geothermalis*.

The term “recombinant bacterium” or “genetically modified bacterium” designates a bacterium that is not found in nature and which contains a modified genome 10 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 encompasses DNA, such as cDNA or gDNA, as well as RNA. The gene may be first 15 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.

The term “expression cassette” denotes a nucleic acid construct comprising a 20 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 comprises an expression cassette. Preferably, the expression vector is a linear or circular 25 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.

The term “control sequences” means nucleic acid sequences necessary for 30 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 transcription terminator. Preferably, the control sequences include a promoter and a 5 transcription terminator.

As used herein, the term “native” 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 a *Deinococcus* bacterium of another species than the recombinant *Deinococcus* bacterium.

10 The term “heterologous” refers to a genetic element or a protein from a non *Deinococcus* origin such as other bacteria, microorganisms, plants, viruses, etc... The terms “native”, “homologous” or “heterologous” may refer to wild-type or mutated genetic element or protein. In some embodiments, these terms refer to genetic elements that are modified, e.g. by mutagenesis, before to be introduced or expressed in the recombinant bacterium

15 of the invention, e.g. to improve one or several characteristics of the encoded protein (e.g. enhanced activity, stability, etc...)

The terms “overexpression”, “enhanced expression” and “increase expression” as used herein, are used interchangeably and mean that the expression of a gene or an enzyme is increased compared to a non modified bacterium, e.g. the wild-type bacterium

20 or the corresponding bacterium that has not been genetically modified in order to enhance the MEP/DXP pathway. Increased expression of an enzyme is usually obtained by increasing expression of the gene encoding said enzyme. In embodiments wherein the gene or the enzyme is not naturally present in the bacterium of the invention, i.e. homologous or heterologous gene or enzyme, the terms “overexpression” and

25 “expression” may be used interchangeably.

The term “MEP pathway”, “MEP/DXP pathway”, “non-mevalonate pathway” or “2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate pathway” as used herein refers to the biosynthetic pathway leading to the formation of IPP and DMAPP from the condensation of pyruvate and D-glyceraldehyde 3-phosphate to 1-

30 deoxy-D-xylulose 5- phosphate (DXP). This pathway involves the following enzymes: 1-deoxy-D-xylulose 5- phosphate synthase (EC 2.2.1.7), 1-deoxy-D-xylulose 5-phosphate reductoisomerase (EC 1.1.1.267), 2-C-methyl-D-erythritol 4-phosphate

cytidyltransferase (EC 2.7.7.60), 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (EC 2.7.1.148), 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (EC 4.6.1.12), 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (EC 1.17.7.1), 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (EC 1.17.1.2), and isopentenyl-diphosphate 5 delta-isomerase (EC 5.3.3.2).

The terms "isoprenoid," "isoprenoid compound," "terpene," "terpene compound," "terpenoid," and "terpenoid compound" are used interchangeably herein, and refer to any compound that is capable of being derived from IPP. The number of C-atoms present in the isoprenoids is typically evenly divisible by five (e.g., C5, C10, C15, C20, C25, C30 10 and C40). Irregular isoprenoids and polyterpenes have been reported, and are also included in the definition of "isoprenoid". Isoprenoid compounds include, but are not limited to, monoterpenes, diterpenes, triterpenes, sesquiterpenes, and carotenoids.

The term terpene synthase encompasses enzymes, complexes, and/or groups of enzymes capable of synthesizing an isoprenoid compound, such as a monoterpene, 15 sesquiterpene, diterpene, triterpene or carotenoid, from one or several precursors, in particular from geranyl diphosphate (GPP), farnesyl diphosphate (FPP), geranylgeranyl diphosphate (GGPP), and any combination of two or more of these. Examples of such terpene synthases include, without limitation, monoterpene synthases, diterpene synthases, and sesquiterpene synthases.

20 The word "pyrophosphate" is used interchangeably herein with "diphosphate".

The term "DXS", "DXP synthase" or "DXPS" refers to the enzyme 1-deoxy-D-xylulose 5-phosphate synthase (EC 2.2.1.7) encoded by the *dxs* gene which catalyzes the condensation of pyruvate and D-glyceraldehyde 3-phosphate to 1-deoxy-D-xylulose 5-phosphate (DXP). The names of gene product, "DXP synthase", "DXS" or "DXPS", are 25 used interchangeably in this application. The DXP synthase activity can be determined by using a radiolabelled substrate as described by Lois et al. (1998) or any other method known by the skilled person.

The terms "DXP reductoisomerase" or "IspC" or "DXR" refer to the enzyme 1-deoxy-D-xylulose 5-phosphate reductoisomerase (EC 1.1.1.267) encoded by the *dxr* or 30 *ispC* gene that catalyzes the simultaneous reduction and isomerization of DXP to 2-C-methyl-D-erythritol-4-phosphate (MEP). The names of the gene, *dxr* or *ispC*, are used

interchangeably in this application. The names of gene product, DXP reductoisomerase or IspC, are used interchangeably in this application.

The term "MCT" or "IspD" or "CMS" and refers to the enzyme 2-C-methyl-D-erythritol 4-phosphate cytidyltransferase (EC 2.7.7.60) encoded by the *ispD* gene that
5 catalyzes the formation of 4-diphosphocytidyl-2-C-methyl-D-erythritol (also named 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol (CDP-ME)) from CTP (cytidine triphosphate) and 2-C-methyl-D-erythritol 4-phosphate (MEP). The names of gene product, MCT, CMS or IspD are used interchangeably in this application.

The term "CMK" or "IspE" and refers to the enzyme 4-diphosphocytidyl-2-C-
10 methyl-D-erythritol kinase, also named 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase, (EC 2.7.1.148) encoded by the *ispE* gene that catalyzes the ATP-dependent phosphorylation of 4-diphosphocytidyl-2C-methyl-D-erythritol (CDP-ME) to 4-diphosphocytidyl-2C-methyl-D-erythritol-2-phosphate (also named 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol 2-phosphate (CDP-MEP). The names of gene
15 product, CMK or IspE are used interchangeably in this application.

The term "IspF", "MECDP-synthase", "MECPP-synthase" or "MECPS" or "MCS" refers to the enzyme 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (EC 4.6.1.12) encoded by the *ispF* gene that catalyzes the conversion of 4-diphosphocytidyl-2-C-methyl-D-erythritol 2-phosphate (CDP-MEP) to 2-C-methyl-D-erythritol 2,4-
20 cyclodiphosphate (ME-CPP or cMEPP) with a corresponding release of cytidine 5-monophosphate (CMP). The names of gene product, IspF, MECDP-synthase, MECPP-synthase, MCS or MECPS are used interchangeably in this application.

The term "IspG" or "HDS" refers to the enzyme 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (EC 1.17.7.1) encoded by the *ispG* gene that is involved in
25 conversion of 2C-methyl-D-erythritol 2,4-cyclodiphosphate (cMEPP) into 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate (HMBPP).

The term "IspH" or "IDS" refers to the enzyme 4-hydroxy-3-methylbut-2-enyl diphosphate reductase, also named hydroxymethylbutenyl pyrophosphate reductase, (EC 1.17.1.2) encoded by the *ispH* gene that converts 1-hydroxy-2-methyl-2-(E)-butenyl 4-
30 diphosphate (HMBPP) into isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP).

The term "IDI", "IPP isomerase" or "isopentenyl pyrophosphate isomerase" refers to the enzyme isopentenyl-diphosphate delta-isomerase (EC 5.3.3.2) encoded by the *fni* or *idi* gene that catalyzes the 1,3-allylic rearrangement of the homoallylic substrate isopentenyl (IPP) to its allylic isomer, dimethylallyl diphosphate (DMAPP). The names 5 of the gene, *fni* or *idi*, are used interchangeably in this application.

The term "mevalonate pathway" or "MEV pathway" is used herein to refer to the biosynthetic pathway that converts acetyl-CoA to IPP. The mevalonate pathway comprises enzymes that catalyze the following steps: (a) condensing two molecules of acetyl-CoA to acetoacetyl-CoA by action of acetyl-CoA C-acetyltransferase (EC 2.3.1.9); 10 (b) condensing acetoacetyl-CoA with acetyl-CoA to form hydroxymethylglutaryl-CoenzymeA (HMG-CoA) by action of hydroxymethylglutaryl-CoA synthase (EC 2.3.3.10); (c) converting HMG-CoA to mevalonate by action of hydroxymethylglutaryl-CoA reductase (NADPH) (EC 1.1.1.34); (d) phosphorylating mevalonate to mevalonate 5-phosphate by action of mevalonate kinase (EC 2.7.1.36); (e) converting mevalonate 5- 15 phosphate to mevalonate 5-pyrophosphate by action of phosphomevalonate kinase (EC 2.7.4.2); and (f) converting mevalonate 5-pyrophosphate to isopentenyl pyrophosphate by action of diphosphomevalonate decarboxylase (EC 4.1.1.33).

The term "IspA", "FDPS", "FPPS" or "FPP synthase" refers to the enzyme farnesyl diphosphate synthase (EC 2.5.1.10, 2.5.1.1) encoded by the *ispA* gene that 20 catalyzes the sequential condensation of isopentenyl pyrophosphate (IPP) with the allylic pyrophosphates, dimethylallyl pyrophosphate (DMAPP), and then with the resultant geranylpyrophosphate (GPP) to the ultimate product farnesyl pyrophosphate (FPP). Optionally, and in preferred embodiments, this enzyme may also exhibit geranylgeranyl diphosphate synthase activity (2.5.1.29). The names of gene product, IspA, FDPS, FPPS 25 or FPP synthase are used interchangeably in this application.

The term "isoprene synthase" refers to an enzyme that catalyzes the formation of isoprene from dimethylallyl diphosphate (EC 4.2.3.27).

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, 30 these terms are used independently from the origin of the enzymes or genes.

As used herein, the term “activity” of an enzyme refers to its function and designates in the context of the invention, the reaction that is catalysed by the enzyme. The enzymatic activity may be measured by any method known by the skilled person.

As used herein, the term “increased activity” or “enhanced activity” or “improved activity” of an enzyme refers to 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. Preferably, the increased activity is an increased intracellular concentration of the enzyme obtained by overexpressing the gene encoding said enzyme.

10 The term “apocarotenoid” as used herein refers to organic compounds derived from carotenoids by oxidative cleavage catalyzed by carotenoid cleavage dioxygenases (CCD). Some of the apocarotenoids are essential and valuable constituents of color, flavor, and aroma in edible plants. Examples of apocarotenoid include, but are not limited to, beta-ionone, alpha-ionone, geranylacetone, pseudoionone and beta-damascenone.

15 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
20 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
25 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
30 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.

Genetic engineering of the MEP pathway

Among the enzymes involved in the MEP pathway, the inventors identified rate-limiting enzymes and showed that their overexpression results in a significant increase in isoprenoid production.

10 Accordingly, in a first aspect, the present invention relates to a recombinant *Deinococcus* bacterium exhibiting enhanced 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate (MEP/DXP) pathway, i.e. a *Deinococcus* bacteria that is genetically modified to have an increased carbon flux to IPP and DMAPP compared to the non modified *Deinococcus* bacterium.

15 In the recombinant *Deinococcus* bacterium of the invention, the activity of at least one enzyme selected from the group consisting of DXS, DXR, IspD, IspE, IspF, IspG, IspH and IPP isomerase, is increased compared to the non modified bacterium. Preferably, at least two, three, four, five, six or seven of these activities are increased. More preferably, all these activities are increased.

20 The activity of these enzymes may be increased due to the overexpression of their encoding genes. Thus, in an embodiment, at least one gene selected from the group consisting of *dxs*, *dxr*, *ispD*, *ispE*, *ispF*, *ispG*, *ispH* and *idi* 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.

25 To increase the expression of a gene, the skilled person can use any known 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 an embodiment, the overexpression is obtained by increasing the copy number 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. Preferably, an expression cassette comprising the gene is integrated into the 5 genome.

Alternatively, the gene may be carried by an expression vector, preferably a plasmid, comprising an expression cassette with the gene of interest. The expression vector may be present in the bacterium in 1 to 5, 20, 100 or 500 copies, depending on the nature of the origin of replication.

10 In another embodiment, the overexpression of the gene is obtained by using a promoter inducing a high level of expression of the gene. For instance, the promoter of an endogenous gene 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 15 or heterologous.

Expression cassettes useful in the present invention comprising at least one gene selected from the group consisting of *dxs*, *dxr*, *ispD*, *ispE*, *ispF*, *ispG*, *ispH* and *idi* genes, operably linked to one or more control sequences, typically comprising a transcriptional promoter and a transcription terminator, that direct the expression of said gene. 20 Preferably, each expression cassette comprises only one gene operably linked to one or more control sequences. In this case, if several genes are overexpressed, several expression cassettes are used, each of them comprising only one gene. Alternatively, expression cassettes useful in the present invention may comprise an operon comprising (i) at least two genes selected from *dxs*, *dxr*, *ispD*, *ispE*, *ispF*, *ispG*, *ispH* and *idi* genes, 25 or (ii) at least one gene selected from *dxs*, *dxr*, *ispD*, *ispE*, *ispF*, *ispG*, *ispH* and *idi* genes, and at least another gene, e.g. a gene encoding a terpene synthase or a FPP synthase.

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 30 in the *Deinococcus* bacterium. The promoter may be a native, homologous or heterologous promoter. Preferred promoters are native or homologous. In this regard,

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 5 (Lecointe *et al*, 2004; Meima *et al*, 2001), or derivatives of such promoters. Preferably, 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 10 be used in the present invention such as, for example, the terminator term116 described 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.

15 In a particular embodiment, the recombinant *Deinococcus* bacterium of the invention comprises: an expression cassette comprising a *dxs* gene operably linked to a strong constitutive promoter; an expression cassette comprising a *dxr* gene operably linked to a strong constitutive promoter; an expression cassette comprising a *ispD* gene operably linked to a strong constitutive promoter; an expression cassette comprising a 20 *ispE* gene operably linked to a strong constitutive promoter; an expression cassette comprising a *ispF* gene operably linked to a strong constitutive promoter; an expression cassette comprising a *ispG* gene operably linked to a strong constitutive promoter; an expression cassette comprising a *ispH* gene operably linked to a strong constitutive promoter; and/or an expression cassette comprising a *idi* gene operably linked to a strong 25 constitutive promoter.

These expression cassettes may be integrated into the genome of the bacterium and/or may be maintained in an episomal form into an expression vector. Preferably, the expression cassettes are integrated into the genome of the bacterium. The bacterium of the invention may overexpress (i) *dxs* (a), *dxr* (b), *ispD* (c), *ispE* (d), *ispF* (e), *ispG* (f), 30 *ispH* (g) or *idi* (h) gene; (ii) one of the following combinations of two genes: a, b ; a, c ; a, d ; a, e ; a, f ; a, g ; a, h ; b, c ; b, d ; b, e ; b, f ; b, g ; b, h ; c, d ; c, e ; c, f ; c, g ; c, h ;

d, e ; d, f ; d, g ; d, h ; e, f ; e, g ; e, h ; f, g ; f, h and g, h ; (iii) one of the following combinations of three genes: a, b, c; a, b, d; a, b, e; a, b, f; a, b, g; a, b, h; a, c, d; a, c, e; a, c, f; a, c, g; a, c, h; a, d, e; a, d, f; a, d, g; a, d, h; a, e, f; a, e, g; a, e, h; a, f, g; a, f, h; a, g, h; b, c, d; b, c, e; b, c, f; b, c, g; b, c, h; b, d, e; b, d, f; b, d, g; b, d, h; b, e, f; b, e, g; b, e, h; b, f, g; b, f, h; b, g, h; c, d, e; c, d, f; c, d, g; c, d, h; c, e, f; c, e, g; c, e, h; c, f, g; c, f, h; c, g, h; d, e, f; d, e, g; d, e, h; d, f, g; d, f, h; d, g, h; e, f, g; e, f, h; e, g, h; and f, g, h; (iv) one of the following combinations of four genes: a, b, c, d; a, b, c, e; a, b, c, f; a, b, c, g; a, b, c, h; a, b, d, e; a, b, d, f; a, b, d, g; a, b, d, h; a, b, e, f; a, b, e, g; a, b, e, h; a, b, f, g; a, b, f, h; a, b, g, h; a, c, d, e; a, c, d, f; a, c, d, g; a, c, d, h; a, c, e, f; a, c, e, g; a, c, e, h; a, c, f, g; a, c, f, h; a, c, g, h; a, d, e, f; a, d, e, g; a, d, e, h; a, d, f, g; a, d, f, h; a, d, g, h; a, e, f, g; a, e, f, h; a, e, g, h; a, f, g, h; b, c, d, e; b, c, d, f; b, c, d, g; b, c, d, h; b, c, e, f; b, c, e, g; b, c, e, h; b, c, f, g; b, c, f, h; b, c, g, h; b, d, e, f; b, d, e, g; b, d, e, h; b, d, f, g; b, d, f, h; b, d, g, h; b, e, f, g; b, e, f, h; b, e, g, h; b, f, g, h; c, d, e, f; c, d, e, g; c, d, e, h; c, d, f, g; c, d, f, h; c, d, g, h; c, e, f, g; c, e, f, h; c, e, g, h; c, f, g, h; d, e, f, g; d, e, f, h; d, e, g, h; d, f, g, h; and e, f, g, h; (v) one of the following combinations of five genes: a, b, c, d, e; a, b, c, d, f; a, b, c, d, g; a, b, c, d, h; a, b, c, e, f; a, b, c, e, g; a, b, c, e, h; a, b, c, f, g; a, b, c, f, h; a, b, c, g, h; a, b, d, e, f; a, b, d, e, g; a, b, d, e, h; a, b, d, f, g; a, b, d, f, h; a, b, d, g, h; a, b, e, f, g; a, b, e, f, h; a, b, e, g, h; a, b, f, g, h; a, c, d, e, f; a, c, d, e, g; a, c, d, e, h; a, c, d, f, g; a, c, d, f, h; a, c, d, g, h; a, c, e, f, g; a, c, e, f, h; a, c, e, g, h; a, c, f, g, h; a, d, e, f, g; a, d, e, f, h; a, d, e, g, h; a, d, f, g, h; a, e, f, g, h; b, c, d, e, f; b, c, d, e, g; b, c, d, e, h; b, c, d, f, g; b, c, d, f, h; b, c, d, g, h; b, c, e, f, g; b, c, e, f, h; b, c, e, g, h; b, c, f, g, h; b, d, e, f, g; b, d, e, f, h; b, d, e, g, h; b, d, f, g, h; b, e, f, g, h; c, d, e, f, g; c, d, e, f, h; c, d, e, g, h; c, d, f, g, h; c, e, f, g, h; and d, e, f, g, h; (vi) one of the following combinations of six genes: a, b, c, d, e, f; a, b, c, d, e, g; a, b, c, d, e, h; a, b, c, d, f, g; a, b, c, d, f, h; a, b, c, d, g, h; a, b, c, e, f, g; a, b, c, e, f, h; a, b, c, e, g, h; a, b, c, f, g, h; a, b, d, e, f, g; a, b, d, e, f, h; a, b, d, e, g, h; a, b, d, f, g, h; a, b, e, f, g, h; a, c, d, e, f, g; a, c, d, e, f, h; a, c, d, e, g, h; a, c, d, f, g, h; a, c, e, f, g, h; a, d, e, f, g, h; b, c, d, e, f, g; b, c, d, e, f, h; b, c, d, e, g, h; b, c, d, f, g, h; b, c, e, f, g, h; b, d, e, f, g, h; and c, d, e, f, g, h; (vii) one of the following combinations of seven genes : a, b, c, d, e, f, g; a, b, c, d, e, f, h; a, b, c, d, e, g, h; a, b, c, d, f, g, h; a, b, c, e, f, g, h; a, b, d, e, f, g, h; a, c, d, e, f, g, h; and b, c, d, e, f, g, h, or (viii) the combination of the eight genes.

In an embodiment, the bacterium overexpresses at least the *ispG* gene and optionally one or several of the *dxs*, *dxr*, *ispD*, *ispE*, *ispF*, *ispH* and *idi* genes.

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

The *dxs* gene may be any gene encoding a 1-deoxyxylulose 5- phosphate synthase, preferably a bacterial gene.

In a preferred embodiment, the *dxs* gene is from a *Deinococcus* bacterium. In particular, the *dxs* gene may be selected from the group consisting of the *dxs* genes from *D. geothermalis* (SEQ ID NO: 1; UniProt accession number: Q1IZP0), *D. yunweiensis* (SEQ ID NO: 3), *D. apachensis* (NCBI Accession number: WP_01958600.1), *D. phoenicis* (GenBank: EYB66726.1; NCBI Accession number: WP_034360373.1; UniProt accession number: A0A016QL59), *D. deserti* (NCBI Accession number: WP_012692944.1; GenBank: ACO45821.1; UniProt accession number: C1D1U7), *D. aquatilis* (NCBI Accession number: WP_026298749.1 or WP_019009670.1), *D. wulumuqiensis* (WP_017869298.1), *D. radiodurans* (UniProt accession number: Q9RUB5; NCBI Accession number: WP_010888114.1), *D. gobiensis* (NCBI Accession number: WP_014684923.1; GenBank: AFD25440.1; UniProt accession number: H8GUB6), *D. sp. 2009* (WP_022801808.1), *D. maricopenis* (NCBI Accession number: WP_013557350.1; UniProt accession number: E8U9V6), *D. peraridilitoris* (NCBI Accession number: WP_015234295.1; UniProt accession number: K9ZXR0), *D. proteolyticus* (NCBI Accession number: WP_013614954.1; UniProt accession number: F0RNL9), *D. radiopugnans* (the sequence encoding the protein set forth in SEQ ID NO: 52; SEQ ID NO: 59), *D. murrayi* (NCBI Accession number: WP_027461316.1), *D. swuensis* (UniProt accession number: A0A0A7KK27 ; NCBI Accession number: WP_039686512.1), *D. solis* (NCBI Accession number: WP_046842902.1) and *D. ficus* (NCBI Accession number: WP_027461725.1). Preferably, the *dxs* gene is selected from the group consisting of the *dxs* genes from *D. geothermalis*, *D. yunweiensis*, *D. apachensis*, *D. phoenicis*, *D. deserti*, *D. aquatilis*, *D. wulumuqiensis*, *D. radiodurans*, *D. gobiensis*, *D. sp. 2009*, *D. maricopenis*, *D. peraridilitoris*, *D. proteolyticus* and *D. radiopugnans*. More preferably, the *dxs* gene is from *D. geothermalis*, *D. yunweiensis* or *D. radiopugnans*, and even more preferably from *D. yunweiensis* or *D. radiopugnans*. Any polypeptide, preferably from a *Deinococcus* bacterium, having at least 70, preferably 80%, more preferably 90%, sequence identity to any of the polypeptides encoded by those genes, preferably to the polypeptide of SEQ ID NO: 2, 4 or 52, more preferably to the polypeptide of SEQ ID NO: 2 or 4, may be used.

The *dxr* gene may be any gene encoding a 1-deoxy-D-xylulose 5-phosphate reductoisomerase, preferably a bacterial gene. In a preferred embodiment, the *dxr* gene is from a *Deinococcus* bacterium. In particular, the *dxr* gene may be selected from the group consisting of the *dxr* genes from *D. geothermalis* (SEQ ID NO: 34; UniProt accession number: Q1IZJ1), *D. yunweiensis* (SEQ ID NO: 36), *D. apachensis* (NCBI Accession number: WP_019584825.1), *D. gobiensis* (NCBI Accession number: WP_014685255.1; UniProt accession number: H8GWW6), *D. phoenicis* (UniProt accession number: A0A016QKH6; GenBank: EYB66645.1), *D. radiodurans* (NCBI accession number: WP_10888147.1; UniProt accession number: Q9RU84), *D. wulumuqiensis* (NCBI accession number: WP_017869266.1), *D. deserti* (NCBI accession number: WP_012693383.1; UniProt accession number: C1CVP3), *D. sp. 2009* (WP_022802132.1), *D. aquatilis* (WP_019009458.1), *D. maricopenis* (NCBI accession number: WP_013557781.1; UniProt accession number: E8UB38), *D. proteolyticus* (NCBI accession number: WP_013614140.1; UniProt accession number: F0RJQ5), *D. peraridilitoris* (NCBI accession number: WP_015237162.1; UniProt accession number: L0A5Z1), *D. swuensis* (UniProt accession number: A0A0A7KF77; GenBank: AIZ44760.1), *D. solis* (WP_046842669.1), *D. marmoris* (WP_029481040.1), *D. ficus* (WP_027462584.1), *D. radiopugnans* (SEQ ID NO: 61) and *D. murrayi* (WP_027460916.1). Preferably, the *dxr* gene is selected from the group consisting of the *dxr* genes from *D. geothermalis*, *D. yunweiensis*, *D. apachensis*, *D. gobiensis*, *D. phoenicis*, *D. radiodurans*, *D. wulumuqiensis*, *D. deserti*, *D. sp. 2009*, *D. aquatilis*, *D. maricopenis*, *D. proteolyticus* and *D. peraridilitoris*. More preferably, the *dxr* gene is from *D. yunweiensis*. Any polypeptide, preferably from a *Deinococcus* bacterium, having at least 70, preferably 80%, more preferably 90%, sequence identity to any of the polypeptides encoded by those genes, preferably to the polypeptide of SEQ ID NO: 35 or 37, may be used.

The *ispD* gene may be any gene encoding a 2-C-methyl-D-erythritol 4-phosphate cytidyltransferase, preferably a bacterial gene. In a preferred embodiment, the *ispD* gene is from a *Deinococcus* bacterium. In particular, the *ispD* gene may be selected from the group consisting of the *ispD* genes from *D. geothermalis* (SEQ ID NO: 17; UniProt accession number: Q1J200), *D. yunweiensis* (SEQ ID NO: 19), *D. apachensis* (NCBI Accession number: WP_019587161.1), *D. phoenicis* (EYB69514.1; UniProt accession number: A0A016QUR2), *D. deserti* (WP_012691946.1; UniProt accession number:

C1CXL7), *D. wulumuqiensis* (WP_017871568.1), *D. radiodurans* (WP_010889228.1; UniProt accession number: Q9RR90), *D. sp. 2009* (WP_022800717.1), *D. peraridilitoris* (WP_015236716.1; UniProt accession number: L0A4P7), *D. aquatilis* (WP_019010949.1 or WP_026298972.1), *D. maricopensis* (WP_013556207.1; UniProt accession number: E8U6L3), *D. proteolyticus* (WP_013615367.1 or WP_041222801.1; GenBank: ADY26759.1; UniProt accession number: F0RKP7), *D. gobiensis* (WP_014686200.1; GenBank: AFD26720.1; UniProt accession number: H8GV45), *D. marmoris* (WP_029480451.1), *D. soli* (AKH17101.1), *D. swuensis* (UniProt accession number: A0A0A7KL82), *D. misasensis* (WP_034344339.1), *D. radiopugnans* (SEQ ID NO: 63) and *D. murrayi* (GenBank: ACO44823.1; WP_027460458.1). Preferably, the *ispD* gene is selected from the group consisting of the *ispD* genes from *D. geothermalis*, *D. yunweiensis*, *D. apachensis*, *D. phoenicis*, *D. deserti*, *D. wulumuqiensis*, *D. radiodurans*, *D. sp. 2009*, *D. peraridilitoris*, *D. aquatilis*, *D. maricopensis*, *D. proteolyticus* and *D. gobiensis*. More preferably, the *ispD* gene is from *D. yunweiensis*.

Any polypeptide, preferably from a *Deinococcus* bacterium, having at least 70, preferably 80%, more preferably 90%, sequence identity to any of the polypeptides encoded by those genes, preferably to the polypeptide of SEQ ID NO: 18 or 20, may be used.

The *ispE* gene may be any gene encoding a 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase, preferably a bacterial gene. In a preferred embodiment, the *ispE* gene is from a *Deinococcus* bacterium. In particular, the *ispE* gene may be selected from the group consisting of the *ispE* genes from *D. geothermalis* (SEQ ID NO: 38; UniProt accession number: Q1J201; NCBI accession number: WP_011529330.1), *D. yunweiensis* (SEQ ID NO: 40), *D. phoenicis* (NCBI Accession number: EYB69513.1; UniProt accession number: A0A016QTX2), *D. apachensis* (WP_019587162.1), *D. aquatilis* (WP_019009083.1), *D. maricopensis* (WP_013556206.1; UniProt accession number: E8U6L2; GenBank: ADV66701.1), *D. deserti* (WP_012691947.1; UniProt accession number: C1CXL8), *D. sp. 2009* (WP_022800716.1), *D. proteolyticus* (WP_013615364.1; UniProt accession number: F0RKH7), *D. wulumuqiensis* (WP_017871569.1), *D. peraridilitoris* (WP_015236715.1 or WP_022800716.1; UniProt accession number: L0A3E0), *D. gobiensis* (WP_014686199.1; UniProt accession number: H8GV44), *D. radiodurans* (WP_10889229.1; UniProt accession number: Q9RR89), *D. swuensis* (A0A0A7KGN1; GenBank: AIZ45322.1), *D. soli* (AKH17102.1), *D. radiopugnans* (SEQ ID NO: 65) and *D. marmoris* (WP_029480453.1). Preferably, the *ispE* gene is

selected from the group consisting of the *ispE* genes from *D. geothermalis*, *D. yunweiensis*, *D. phoenicis*, *D. apachensis*, *D. aquatilis*, *D. maricopenis*, *D. deserti*, *D. sp. 2009*, *D. proteolyticus*, *D. wulumuqiensis*, *D. peraridilitoris*, *D. gobiensis* and *D. radiodurans*. More preferably, the *ispE* gene is from *D. yunweiensis*. Any polypeptide, preferably from a *Deinococcus* bacterium, having at least 70, preferably 80%, more preferably 90%, sequence identity to any of the polypeptides encoded by those genes, preferably to the polypeptide of SEQ ID NO: 39 or 41, may be used.

The *ispF* gene may be any gene encoding a 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase, preferably a bacterial gene. In a preferred embodiment, the *ispF* gene is from a *Deinococcus* bacterium. In particular, the *ispF* gene may be selected from the group consisting of the *ispF* genes from *D. geothermalis* (SEQ ID NO: 21; UniProt accession number: Q1J2A8; WP_011529223.1), *D. yunweiensis* (SEQ ID NO: 23), *D. phoenicis* (NCBI Accession number: EYB69383.1, WP_034353365.1; UniProt accession number: A0A016QTI4), *D. apachensis* (WP_019587119.1), *D. sp. 2009* (WP_022800431.1), *D. wulumuqiensis* (WP_017871252.1), *D. maricopenis* (WP_013558001.1; UniProt accession number: E8UBQ8), *D. deserti* (WP_012692194.1; UniProt accession number: C1CZ33), *D. aquatilis* (WP_019009115.1), *D. radiodurans* (WP_010886876.1 UniProt accession number: Q9RXS6), *D. proteolyticus* (WP_013615320.1; UniProt accession number: F0RKD3), *D. gobiensis* (WP_014686259.1; UniProt accession number: H8GVA4), *D. peraridilitoris* (WP_015234905.1), *D. swuensis* (A0A0A7KKV6), *D. radiopugnans* (SEQ ID NO: 67) and *D. murrayi* (WP_027461473.1). Preferably, the *ispF* gene is selected from the group consisting of the *ispF* genes from *D. geothermalis*, *D. yunweiensis*, *D. phoenicis*, *D. apachensis*, *D. sp. 2009*, *D. wulumuqiensis*, *D. maricopenis*, *D. deserti*, *D. aquatilis*, *D. radiodurans*, *D. proteolyticus*, *D. gobiensis* and *D. peraridilitoris*. More preferably, the *ispF* gene is from *D. yunweiensis*. Any polypeptide, preferably from a *Deinococcus* bacterium, having at least 70, preferably 80%, more preferably 90%, sequence identity to any of the polypeptides encoded by those genes, preferably to the polypeptide of SEQ ID NO: 22 or 24, may be used.

The *ispG* gene may be any gene encoding a 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase, preferably a bacterial gene. In a preferred embodiment, the *ispG* gene is from a *Deinococcus* bacterium. In particular, the *ispG* gene may be selected from

the group consisting of the *ispG* genes from *D. geothermalis* (SEQ ID NO: 25), *D. yunweiensis* (SEQ ID NO: 27), *D. apachensis* (NCBI Accession number: WP_019584739.1 or WP_040382265.1), *D. phoenicis* (EYB67750.1), *D. aquatilis* (WP_019008200.1), *D. sp. 2009* (WP_022801633.1), *D. gobiensis* (WP_014684632.1),
 5 *D. radiodurans* (WP_010887031.1), *D. wulumuqiensis* (WP_017871942.1), *D. deserti* (WP_012693251.1), *D. proteolyticus* (WP_013615171.1), *D. maricopensis* (WP_013556907.1; EYB67750.1), *D. peraridilitoris* (WP_015237094.1), *D. radiopugnans* (SEQ ID NO: 69) and *D. murrayi* (WP_027460867.1). Preferably, the *ispG* gene is selected from the group consisting of the *ispG* genes from *D. geothermalis*, *D.*
 10 *yunweiensis*, *D. apachensis*, *D. phoenicis*, *D. aquatilis*, *D. sp. 2009*, *D. gobiensis*, *D. radiodurans*, *D. wulumuqiensis*, *D. deserti*, *D. proteolyticus*, *D. maricopensis* and *D. peraridilitoris*. More preferably, the *ispG* gene is from *D. yunweiensis*. Any polypeptide, preferably from a *Deinococcus* bacterium, having at least 70, preferably 80%, more preferably 90%, sequence identity to any of the polypeptides encoded by those genes,
 15 preferably to the polypeptide of SEQ ID NO: 26 or 28, may be used.

The *ispH* gene may be any gene encoding a 4-hydroxy-3-methylbut-2-enyl diphosphate reductase, preferably a bacterial gene. In a preferred embodiment, the *ispH* gene is from a *Deinococcus* bacterium. In particular, the *ispH* gene may be selected from the group consisting of the *ispH* genes from *D. geothermalis* (SEQ ID NO: 42), *D.*
 20 *yunweiensis* (SEQ ID NO: 44), *D. phoenicis* (NCBI Accession number: EYB6823.1; WP_034356434.1), *D. apachensis* (WP_019585890.1), *D. sp. 2009* (WP_022801928.1), *D. gobiensis* (WP_014685178.1), *D. aquatilis* (WP_019007893.1), *D. radiodurans* (WP_10888795.1), *D. deserti* (WP_012693059.1; GeneBank: ACO45936.1), *D. peraridilitoris* (WP_015236935.1), *D. proteolyticus* (WP_013614360.1), *D.*
 25 *maricopensis* (WP_013557691.1), *D. radiopugnans* (SEQ ID NO: 71) and *D. wulumuqiensis* (WP_017869851.1). Preferably, the *ispH* gene is selected from the group consisting of the *ispH* genes from *D. geothermalis*, *D. yunweiensis*, *D. apachensis*, *D. phoenicis*, *D. aquatilis*, *D. sp. 2009*, *D. gobiensis*, *D. radiodurans*, *D. wulumuqiensis*, *D. deserti*, *D. proteolyticus*, *D. maricopensis* and *D. peraridilitoris*. More preferably, the
 30 *ispH* gene is from *D. yunweiensis*. Any polypeptide, preferably from a *Deinococcus* bacterium, having at least 70, preferably 80%, more preferably 90%, sequence identity to any of the polypeptides encoded by those genes, preferably to the polypeptide of SEQ ID NO: 43 or 45, may be used.

The *idi* gene may be any gene encoding an isopentenyl-diphosphate delta-isomerase, preferably a bacterial gene. In a preferred embodiment, the *idi* gene is from a *Deinococcus* bacterium. In particular, the *idi* gene may be selected from the group consisting of the *idi* genes from *D. geothermalis* (SEQ ID NO: 29), *D. yunweiensis* (SEQ ID NO: 31), *D. apachensis* (NCBI Accession number: WP_019585561.1), *D. phoenicis* (EYB67918.1), *D. aquatilis* (WP_019010639.1 or WP_040380849.1), *D. sp. 2009* (WP_022802239.1), *D. deserti* (WP_012692934.1), *D. radiodurans* (Q9RVE2.3), *D. maricopenis* (WP_013556085.1), *D. wulumuqiensis* (WP_017870894.1), *D. gobiensis* (WP_014685602.1 or WP_043802116.1), *D. radiopugnans* (SEQ ID NO: 73) and *D. peraridilitoris* (WP_015236653.1). Preferably, the *idi* gene is selected from the group consisting of the *idi* genes from *D. geothermalis*, *D. yunweiensis*, *D. apachensis*, *D. phoenicis*, *D. aquatilis*, *D. sp. 2009*, *D. gobiensis*, *D. radiodurans*, *D. wulumuqiensis*, *D. deserti*, *D. maricopenis* and *D. peraridilitoris*. More preferably, the *idi* gene is from *D. yunweiensis*. Any polypeptide, preferably from a *Deinococcus* bacterium, having at least 70, preferably 80%, more preferably 90%, sequence identity to any of the polypeptides encoded by those genes, preferably to the polypeptide of SEQ ID NO: 30 or 32, may be used.

In a preferred embodiment, the bacterium overexpresses DXS and IDI encoding genes. Preferably, the overexpressed DXS is an improved DXS enzyme as described below.

Improved MEP enzymes

The *dxs*, *drx*, *ispD*, *ispE*, *ispF*, *ispG*, *ispH* and *idi* genes used in the present invention may also encode 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. Such improved enzymes may be obtained according to any techniques known by the skilled person. In particular, improved DXP synthases, more particularly improved *Deinococcus* DXP synthases, may be obtained according to the technique disclosed in the patent application WO 2012/052171.

Thus, in an embodiment, the recombinant *Deinococcus* bacterium comprises a *dxs* gene encoding an improved DXP synthase, preferably an improved *Deinococcus* DXP

synthase, obtained by mutating a DXP synthase in order to obtain an asparagine at position corresponding to position 222 of SEQ ID NO: 52 (or at position corresponding to position 216 of SEQ ID NO: 4 or at position corresponding to position 219 of SEQ ID NO: 2) and/or a cysteine at position corresponding to position 244 of SEQ ID NO: 52 (or at position corresponding to position 238 of SEQ ID NO: 4 or at position corresponding to position 241 of SEQ ID NO: 2). Preferably, the recombinant *Deinococcus* bacterium comprises a *dxs* gene encoding an improved DXP synthase obtained by mutating a DXP synthase in order to obtain a cysteine at position corresponding to position 244 of SEQ ID NO: 52 (or at position corresponding to position 238 of SEQ ID NO: 4 or at position corresponding to position 241 of SEQ ID NO: 2).

Methods to identify residues corresponding to position 222 or 244 are well known by the skilled person and detailed in the patent application WO 2012/052171. In particular, these residues may be identified using a sequence alignment computer program such as ClustalW, MUSCLE or programs using the Needleman and Wunsch algorithm.

In a preferred embodiment, the recombinant *Deinococcus* bacterium comprises a gene encoding an improved DXP synthase from *D. radiopugnans* having the following substitutions: K222N (SEQ ID NO: 6), R244C (SEQ ID NO: 8) or K222N/R244C (SEQ ID NO: 10). The sequences of the genes encoding these enzymes are presented in SEQ ID NO: 5, 7 and 9, respectively. More preferably, the recombinant *Deinococcus* bacterium comprises a gene encoding R244C mutant of the DXP synthase from *D. radiopugnans* (SEQ ID NO: 8).

In another preferred embodiment, the recombinant *Deinococcus* bacterium comprises a gene encoding an improved DXP synthase from *D. yunweiensis* having the following substitutions: K216N (SEQ ID NO: 12), R238C (SEQ ID NO: 14) or K216N/R238C (SEQ ID NO: 16). The sequences of the genes encoding these enzymes are presented in SEQ ID NO: 11, 13 and 15, respectively. More preferably, the recombinant *Deinococcus* bacterium comprises a gene encoding R238C mutant of the DXP synthase from *D. yunweiensis* (SEQ ID NO: 14).

In a further preferred embodiment, the recombinant *Deinococcus* bacterium comprises a gene encoding an improved DXP synthase from *D. geothermalis* (SEQ ID

NO: 2) having the following substitutions: K219N (SEQ ID NO: 54), R241C (SEQ ID NO: 56) or K219N/R241C (SEQ ID NO: 58). More preferably, the recombinant *Deinococcus* bacterium comprises a gene encoding R241C mutant of the DXP synthase from *D. geothermalis*.

5 The gene encoding the improved DXP synthase may be overexpressed in the recombinant bacterium. Preferably, this gene is integrated in the genome of the bacterium under the control of constitutive or inducible promoter, more preferably under the control of a strong constitutive promoter. This gene can replace the endogenous *dxs* gene or may be integrated in another place to increase the copy number of the *dxs* gene.

10 Thus, in another aspect, the present invention also relates to a recombinant *Deinococcus* bacterium comprising an improved (or mutated) DXP synthase, preferably an improved *Deinococcus* DXP synthase, comprising an asparagine at position corresponding to position 222 of SEQ ID NO:52 and/or a cysteine at position corresponding to position 244 of SEQ ID NO:52. Preferably, the recombinant
15 *Deinococcus* bacterium comprises an improved (or mutated) DXP synthase comprising a cysteine at position corresponding to position 244 of SEQ ID NO:52. The corresponding position can be easily identified by the skilled person using well-known alignment algorithms. In particular, the improved DXP synthase may be any of the improved DXS disclosed above.

20 Optionally, at least one gene selected from the group consisting of *dxs*, *dxr*, *ispD*, *ispE*, *ispF*, *ispG*, *ispH* and *idi*, is overexpressed. Preferably, at least the IDI encoding gene is overexpressed. More preferably, at least one *dxs* gene and one *idi* gene are overexpressed.

25 All embodiments described herein for the other aspects of the invention are also encompassed in this aspect. In particular, the recombinant *Deinococcus* bacterium may further exhibit (i) an enhanced MEP pathway as described above, (ii) a heterologous MEV pathway, (iii) an increase of the FPP synthase activity, (iv) inactivation of the lycopene beta-cyclase and/or (v) expression of a heterologous terpene or isoprene synthase as described herein.

Heterologous MEV pathway

In another aspect, the recombinant *Deinococcus* bacterium of the invention comprises a heterologous MEV pathway, i.e. expresses heterologous genes encoding hydroxymethylglutaryl-CoA synthase (EC 2.3.3.10), hydroxymethylglutaryl-CoA reductase (NADPH) (EC 1.1.1.34), mevalonate kinase (EC 2.7.1.36), phosphomevalonate kinase (EC 2.7.4.2), and diphosphomevalonate decarboxylase (EC 4.1.1.33).

Preferably, heterologous genes encode enzymes from plants, bacteria or yeasts, more preferably from *Saccharomyces cerevisiae*. In a particular embodiment, the recombinant *Deinococcus* bacterium comprises genes encoding *Saccharomyces cerevisiae* hydroxymethylglutaryl-CoA synthase (Uniprot/Swissprot: P54839), hydroxymethylglutaryl-CoA reductase (NADPH) (Uniprot/Swissprot: P12683), mevalonate kinase (Genbank: NP_013935.1), phosphomevalonate kinase (Genbank: NP_013947.1), and diphosphomevalonate decarboxylase (Genbank: CAA66158.1).

Deinococcus bacteria exhibit endogenous acetyl-CoA C-acetyltransferase activity. However, in some embodiment, the recombinant *Deinococcus* bacterium may further comprise a heterologous gene encoding an acetyl-CoA C-acetyltransferase. This gene can replace the endogenous gene or may be integrated in another place to increase the copy number of this gene. This heterologous gene may be from plants, bacteria or yeasts. In a preferred embodiment, the recombinant *Deinococcus* bacterium further comprises a gene encoding a *Saccharomyces cerevisiae* acetyl-CoA C-acetyltransferase (Genbank: NP_015297.1).

All embodiments described herein for the other aspects of the invention are also encompassed in this aspect. In particular, the recombinant *Deinococcus* bacterium may further exhibit (i) an enhanced MEP pathway as described above, optionally with an improved DXP synthase, (ii) an increase of the FPP synthase activity, (iii) inactivation of the lycopene beta-cyclase and/or (iv) expression of a heterologous terpene or isoprene synthase as described herein.

Heterologous terpene synthases

The present application also demonstrates that *Deinococcus* bacteria, and in particular recombinant *Deinococcus* bacteria as described above, may be efficiently used

to produce heterologous isoprenoid compounds via the expression of heterologous terpene synthases. Thus, in a further aspect, the present invention relates to a recombinant *Deinococcus* bacteria comprising a gene encoding a heterologous terpene synthase.

All embodiments described herein for the other aspects of the invention are also encompassed in this aspect. In particular, the recombinant *Deinococcus* bacterium may further exhibit (i) an enhanced MEP pathway as described above, optionally with an improved DXP synthase, (ii) a heterologous MEV pathway, (iii) an increase of the FPP synthase activity, (iv) inactivation of the lycopene beta-cyclase and/or (v) expression of a heterologous isoprene synthase as described herein.

Preferably, the heterologous terpene synthase is selected from the group consisting of monoterpene synthases, diterpene synthases, triterpene synthases and sesquiterpene synthases, more preferably from monoterpene and sesquiterpene synthases. The recombinant *Deinococcus* bacterium may express one or several heterologous terpene synthases, in particular one or several monoterpene synthases and/or one or several sesquiterpene synthases.

Each heterologous terpene synthase is preferably expressed from an expression cassette as described above, comprising the gene encoding said enzyme and integrated into the genome of the bacterium. Preferably, the gene is under the control of a constitutive promoter, more preferably a strong constitutive promoter.

Heterologous monoterpene synthases

In a particular embodiment, the recombinant bacterium of the invention is a *Deinococcus* bacterium modified via the expression of a heterologous monoterpene synthase to produce a monoterpene or monoterpenoid. Example of monoterpene synthases that can be expressed in the recombinant *Deinococcus* bacterium of the invention include, but are not limited to, geraniol synthases (EC 3.1.7.3), linalool synthases such as 3S-linalool synthases (EC 4.2.3.25) and R-linalool synthases (EC 4.2.3.26), 1,8-cineole synthase (EC 4.2.3.108), myrcene synthases (EC 4.2.3.15), 4S-limonene synthases (EC 4.2.3.16), R-limonene synthases (EC 4.2.3.20), α -pinene synthases (EC 4.2.3.119), β -pinene synthases (EC 4.2.3.120), (-)-endo-fenchol synthases (EC 4.2.3.10), and (-)- α -terpineol synthases (EC 4.2.3.111), and any combinations thereof.

In an embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous geraniol synthase, i.e. an enzyme that catalyzes the formation of geraniol from geranyl diphosphate. Examples of heterologous geraniol synthases include, but are not limited to, geraniol synthases from *Ocimum basilicum* (SEQ ID NO: 49; 5 GenBank accession number: AAR11765, Zhou et al., 2013; Liu et al., 2013; Lijima et al., 2004), *Valeriana officinalis* (AHE41084, Dong et al., 2013), *Lippia dulcis* (Dong et al., 2013), *Perilla citriodora* (AAY88965 ; Sugiura et al., 2011), *Perilla frutescens* (ABB30218 ; Sharkey et al., 2005), *Perilla setoyensis*, (ACN42010.1), *Cinnamomum tenuipilum* (CAD29734), *Phyla dulcis* (ADK62524.1), *Olea europaea* (AFI47926.1), 10 *Vitis vinifera* (ADR74217), *Cicer arietinum* (XP_004487383.1), *Picrorhiza kurrooa* (AHX97739.1), *Citrus jambhiri* (BAM29049.1), *Cinnamomum tenuipile* (CAD29734.2; Yang et al., 2005) and *Catharanthus roseus* (AFD64744.1). Any polypeptide exhibiting a geraniol synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to any of these enzymes may be used, in particular variants of these enzymes 15 having increased catalytic activity, specificity for the substrate, and /or stability. In a particular embodiment, the heterologous geraniol synthase is selected from the group consisting of geraniol synthases from *Ocimum basilicum*, *Valeriana officinalis*, *Phyla dulcis*, *Olea europaea*, *Vitis vinifera*, *Cicer arietinum* and *Catharanthus roseus*. More preferably, the heterologous geraniol synthase is the geraniol synthase of *Ocimum* 20 *basilicum* (SEQ ID NO: 49), *Cicer arietinum* or *Phyla dulcis*. Even more preferably, the geraniol synthase is the geraniol synthase of *Ocimum basilicum*. The nucleotide sequence of the gene encoding this geraniol synthase may be as set forth in SEQ ID NO: 48.

Optionally, the recombinant bacterium comprising a gene encoding a heterologous geraniol synthase, further comprises a geraniol reductase catalyzing the 25 reduction of geraniol to citronellol (EC 1.6.99.1). Examples of heterologous geraniol reductases include, but are not limited to, the old yellow enzyme 2 (OYE2) from *S. cerevisiae* (Q03558.3) and the homologous from the plant *Hevea brasiliensis*, a 12-oxophytodienoate reductase (OPR), HbOPR (DQ004685.1).

In another embodiment, the recombinant bacterium of the invention comprises a 30 gene encoding a heterologous linalool synthase. The linalool synthase may be a 3S-linalool synthase, i.e. an enzyme that catalyzes the formation of S-linalool from geranyl diphosphate, or a R-linalool synthase, i.e. an enzyme that catalyzes the formation of R-

linalool from geranyl diphosphate. Examples of heterologous linalool synthases include, but are not limited to, linalool synthases from *Streptomyces clavuligerus* (Nakano et al., 2011), *Lemon Myrtle* (Sugiura et al., 2011), *Perilla citriodora* (GenBank accession number AHY39266.1; Sugiura et al., 2011), *Myzus citrate* (Sugiura et al., 2011), *Clarkia*
 5 *breweri* (AAD19838; AAD19840; AAC49395, Rico et al., 2010; Herreo et al., 2008; Dudareva et al., 1996; Pichersky et al., 1995), *Clarkia concinna* (AAD19839), *Lavandula angustivolia* (ABB73045.1; Landmann et al., 2007) *Vitis vinifera* (Martin et al., 2010), *Arabidopsis lyrata* subsp. *lyrata* (EFH62782.1; XP_002886523.1), *Oryza sativa* (EU596453), *Cereus peruvianus* (Sitrit et al., 2004), *Mentha citrata* (AAL99381; Crowell
 10 et al., 2002), *Artemisia annua* (AAF13357; AAF13356; Jia et al., 1999), *Ocimum basilicum* (AAV63789; Lijima et al., 2004), *Arabidopsis thaliana* (AAO85533; Chen et al., 2003), *Fragaria ananassa* (CAD57081; CAD57106; Aharoni et al., 2004), *Medicago truncatula* (XP_003593502.1), *Antirrhinum majus* (ABR24418), *Lycopersicon esculentum* (AAX69063), *Picea abies* (AAS47693), *Perilla frutescens* var. *hirtella*
 15 (ACN42011; Masumoto et al., 2010), *Coriandrum sativum* (AHC54051; Galata et al., 2014), *Cinnamomum osmophloeum* (AFQ20812.1), *Theobroma cacao* (EOY20311.1), *Morus notabilis* (EXC23176.1) and *Coffea Arabica* (Del Terra et al., 2013). Any polypeptide exhibiting a linalool synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to any of these enzymes may be used, in particular
 20 variants of these enzymes having increased catalytic activity, specificity for the substrate, and /or stability. In a particular embodiment, the heterologous linalool synthase is selected from the group consisting of linalool synthases from *Clarkia concinna*, *Medicago truncatula*, *Perilla frutescens* var. *hirtella*, *Coriandrum sativum*, *Lavandula angustivolia*, *Clarkia breweri*, *Cinnamomum osmophloeum*, *Theobroma cacao*, *Morus notabilis*, and
 25 *Coffea Arabica*. More preferably, the heterologous linalool synthase is a linalool synthase of *Perilla frutescens* or *Clarkia breweri*, even more preferably of *Perilla frutescens*.

In an embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous myrcene synthase, i.e. an enzyme that catalyzes the formation of myrcene from geranyl diphosphate. Examples of heterologous myrcene synthases
 30 include, but are not limited to, myrcene synthases from *Abies grandis* (GenBank accession number: AAB71084), *Antirrhinum majus* (AAO41727; AAO41726), *Arabidopsis thaliana* (NP_189209; AAG09310), *Lycopersicon esculentum* (AAX69064), *Ocimum basilicum* (AAV63791), *Perilla frutescens* (AAF76186), *Picea abies*

(AAS47696), *Morus notabilis* (EXB97316.1), *Medicago truncatula* (AES67305.1) and *Quercus ilex* (CAC41012). Any polypeptide exhibiting a myrcene synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to any of these enzymes may be used, in particular variants of these enzymes having increased catalytic activity, 5 specificity for the substrate, and /or stability. In a particular embodiment, the heterologous linalool synthase is selected from the group consisting of myrcene synthases from *Abies grandis*, *Picea abies*, *Morus notabilis* and *Medicago truncatula*.

In another embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous limonene synthase. The limonene synthase may be a 4S- 10 limonene synthase, i.e. an enzyme that catalyzes the formation of (-)-S-limonene from geranyl diphosphate, or a R-limonene synthase, i.e. an enzyme that catalyzes the formation of (+)-R-limonene from geranyl diphosphate. Examples of heterologous limonene synthases include, but are not limited to, limonene synthases from *Mentha spicata* (GenBank accession number: AAC37366), *Mentha longifolia* (AAD50304.1), 15 *Abies grandis* (AAB70907; AAF61455), *Agastache rugosa* (AAL17636), *Cannabis sativa* (ABI21837), *Citrus limon* (AAM53944; AAM53946), *Citrus unshiu* (BAD27256; BAD27257), *Lavandula angustifolia* (ABB73044), *Perilla frutescens* (AAG31438), *Picea abies* (AAS47694), *Picea sitchensis* (ABA86248) and *Schizonepeta tenuifolia* (AAG01140). Any polypeptide exhibiting a limonene synthase activity and having at 20 least 70%, 80%, preferably 90%, sequence identity to any of these enzymes may be used, in particular variants of these enzymes having increased catalytic activity, specificity for the substrate, and /or stability. In a particular embodiment, the heterologous limonene synthase is from *Mentha spicata* or *Mentha longifolia*, preferably *Mentha longifolia*.

In another embodiment, the recombinant bacterium of the invention comprises a 25 gene encoding a heterologous cineole synthase. Examples of heterologous cineole synthases include, but are not limited to, cineole synthases from *Streptomyces clavuligerus* (GenBank: EDY47508.1; Nakano et al., 2011), *Solanum lycopersicum* (GenBank: AEM05857.1), *Rosmarinus officinalis* (GenBank: AFZ41794.1), *Salvia fruticosa* (ABH07677.1) and *Arabidopsis thaliana* (AEE77075.1; AEE77074.1). Any 30 polypeptide exhibiting a cineol synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to any of these enzymes may be used, in particular variants of these enzymes having increased catalytic activity, specificity for the substrate, and/or

stability. In a particular embodiment, the heterologous cineol synthase is from *Streptomyces clavuligerus*.

Optionally, the recombinant bacterium of the invention comprising a heterologous limonene synthase may further comprise one or several additional enzymes belonging to the pathways converting (i) S-limonene to (-)-carvone, (ii) S-limonene to menthone, menthol, neomenthol or isomenthone, or (iii) R-limonene to (+) carvone. Thus, in a particular embodiment, the recombinant *Deinococcus* bacterium expresses a heterologous limonene synthase and one or several additional heterologous enzymes selected from the groups consisting of (i) (S)-limonene 6-monooxygenase (EC 1.14.13.48) and carveol dehydrogenase (EC1.1.1.243); (ii) (S)-limonene 3-monooxygenase (EC1.14.13.47), isopiperitenol dehydrogenase (EC 1.1.1.223), (-)-isopiperitenone reductase (EC1.3.1.82), (+)-cis-isopulegone isomerase, (+)-pulegone reductase (EC 1.3.1.81), (-)-menthol dehydrogenase (EC 1.1.1.207) and (+)-neomenthol dehydrogenase (EC 1.1.1.208); and (iii) (R)-limonene 6-monooxygenase (EC 1.14.13.80) and (+)-trans-carveol dehydrogenase (EC 1.1.1.243).

In another embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous pinene synthase. The pinene synthase may be a α -pinene synthase, i.e. an enzyme that catalyzes the formation of α -pinene from geranyl diphosphate, or a β -pinene synthase, i.e. an enzyme that catalyzes the formation of β -pinene from geranyl diphosphate. Examples of heterologous pinene synthases include, but are not limited to, pinene synthases from *Abies grandis* (GenBank accession number: AAB71085), *Artemisia annua* (AAK58723), *Cannabis sativa* (ABI21838), *Citrus limon* (AAM53945), *Citrus unshiu* (BAD27260), *Fragaria vesca* (CAD57092), *Picea abies* (AAS47692), *Picea sitchensis* (AAP72020) and *Pseudotsuga menziesii* (AAX07267). Any polypeptide exhibiting a pinene synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to any of these enzymes may be used, in particular variants of these enzymes having increased catalytic activity, specificity for the substrate, and /or stability. In a particular embodiment, the heterologous pinene synthase is from *Artemisia annua*.

In an embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous (-)-endo-fenchol synthase, i.e. an enzyme that catalyzes the formation of fenchol from geranyl diphosphate. Examples of heterologous (-)-endo-

fenchol synthases include, but are not limited to, the (-)-endo-fenchol synthase from *Ocimum basilicum* (GenBank accession number: AAV63790). Any polypeptide exhibiting an endo-fenchol synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to this enzyme may be used, in particular variants of this enzyme
5 having increased catalytic activity, specificity for the substrate, and /or stability.

In an embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous (-)- α -terpineol synthase, i.e. an enzyme that catalyzes the formation of α -terpineol from geranyl diphosphate. Examples of heterologous (-)- α -terpineol synthases include, but are not limited to, (-)- α -terpineol synthases from *Vitis*
10 *vinifera* (GenBank accession number: AAS79351; AAS79352), *Zea mays* (AAL59230; ABR09292), *Santalum album* (ACF24767), *Pinus tadea* (AAO61227) and *Magnolia grandiflora* (ACC66282). Any polypeptide exhibiting an α -terpineol synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to this enzyme may be used, in particular variants of this enzyme having increased catalytic activity, specificity
15 for the substrate, and /or stability. In a particular embodiment, the (-)- α -terpineol synthase is from *Vitis vinifera*.

Heterologous sesquiterpene synthases

In another embodiment, the recombinant bacterium of the invention is a *Deinococcus* bacterium modified via the expression of a heterologous sesquiterpene
20 synthase to produce a sesquiterpenoid. Example of sesquiterpene synthases that can be expressed in the recombinant *Deinococcus* bacterium of the invention include, but are not limited to, (+)-epi- α -bisabolol synthase (EC 4.2.3.138), germacrene A synthase (EC 4.2.3.23), (E,E)-germacrene B synthase (EC 4.2.3.71), germacrene C synthase (EC 4.2.3.60), (-)-germacrene D synthase (EC 4.2.3.75), valencene synthase (EC 4.2.3.73),
25 (3S, 6E)-nerolidol synthase (EC 4.2.3.48), epi-cedrol synthase (EC 4.2.3.39), patchoulol synthase (EC 4.2.3.70), santalene synthase (EC 4.2.3.50) and δ -cadinene synthase (EC 4.2.3.13).

In an embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous (+)-epi- α -bisabolol synthase, i.e. an enzyme that catalyzes
30 the formation of (+)-epi- α -bisabolol from (2E,6E)-farnesyl diphosphate. Examples of (+)-epi- α -bisabolol synthases include, but are not limited to, (+)-epi- α -bisabolol

synthases from *Streptomyces citricolor* (GenBank accession number: BAL14867.1; Nakano et al., 2011) and *Phyla dulcis* (Attia et al., 2012). Any polypeptide exhibiting (+)-epi-alpha-bisabolol synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to this enzyme may be used, in particular variants of this enzyme having
5 increased catalytic activity, specificity for the substrate, and /or stability. In a particular embodiment, the (+)-epi-alpha-bisabolol synthase is from *Streptomyces citricolor*.

In an embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous germacrene A synthase, i.e. an enzyme that catalyzes the cyclization of farnesyl diphosphate (FPP) to the sesquiterpene germacrene A. Examples
10 of heterologous germacrene A synthases include, but are not limited to, germacrene A synthases from *Artemisia annua* (GenBank accession number: ABE03980), *Cichorium intybus* (AAM21658; AAM21659), *Ixeris dentate* (AAL92481), *lactuca sativa* (AAM11626); AAM11627), *Pogostemon cablin* (AAS86321) and *Solidago Canadensis* (CAC36896). Any polypeptide exhibiting a germacrene A synthase activity and having
15 at least 80%, preferably 90%, sequence identity to this enzyme may be used, in particular variants of this enzyme having increased catalytic activity, specificity for the substrate, and /or stability. In a particular embodiment, the germacrene A synthase is selected from *Artemisia annua* and *Solidago Canadensis*.

In an embodiment, the recombinant bacterium of the invention comprises a gene
20 encoding a heterologous germacrene B synthase, i.e. an enzyme that catalyzes the cyclization of farnesyl diphosphate (FPP) to the sesquiterpene germacrene B. Examples of heterologous germacrene B synthases include, but are not limited to, germacrene B synthase from *Lycopersicon hirsutum* (GenBank accession number: AAG41891). Any polypeptide exhibiting an germacrene B synthase activity and having at least 70%, 80%,
25 preferably 90%, sequence identity to this enzyme may be used, in particular variants of this enzyme having increased catalytic activity, specificity for the substrate, and /or stability.

In an embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous germacrene C synthase, i.e. an enzyme that catalyzes the
30 cyclization of farnesyl diphosphate (FPP) to the sesquiterpene germacrene C. Examples of heterologous germacrene C synthases include, but are not limited to, germacrene C synthase from *Lycopersicon esculentum* (GenBank accession number: AAC39432). Any

polypeptide exhibiting an germacrene B synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to this enzyme may be used, in particular variants of this enzyme having increased catalytic activity, specificity for the substrate, and /or stability.

5 In an embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous germacrene D synthase, i.e. an enzyme that catalyzes the cyclization of farnesyl diphosphate (FPP) to the sesquiterpene germacrene D. Examples of heterologous germacrene D synthases include, but are not limited to, germacrene D synthases from *Lycopersicon hirsutum* (GenBank accession number: AAG41892),
10 *Ocimum basilicum* (AAV63786), *Pogostemon cablin* (AAS86320, AAS86322), *Populus trichocarpa x Populus deltoides* (AAR99061), *Solidago Canadensis* (AAR31144, AAR31145), *Vitis vinifera* (AAS66357) and *Zingiber officinale* (AAX40665). Any polypeptide exhibiting a germacrene D synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to any of these enzymes may be used, in particular
15 variants of these enzymes having increased catalytic activity, specificity for the substrate, and /or stability.

In an embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous valencene synthase, i.e. an enzyme that catalyzes the formation of valencene from farnesyl diphosphate (FPP). Examples of heterologous valencene
20 synthases include, but are not limited to, valencene synthases from *Vitis vinifera* (UniProt accession number Q6Q3H2) and *Citrus sinensis* (Q71MJ3). Any polypeptide exhibiting a valencene synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to any of these enzymes may be used, in particular variants of these enzymes having increased catalytic activity, specificity for the substrate, and /or stability.

25 In an embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous patchoulol synthase, i.e. an enzyme that catalyzes the formation of patchoulol from farnesyl diphosphate (FPP). Examples of heterologous patchoulol synthases include, but are not limited to, patchoulol synthase from *Pogostemon cablin* (UniProt accession number Q49SP3) which produces additional sesquiterpene products,
30 including alpha- and beta-patchoulene, alpha-bulnesene, seychellene, pogostol and alpha-guaiaene. Any polypeptide exhibiting a patchoulol synthase activity and having at least

70%, 80%, preferably 90%, sequence identity to this enzyme may be used, in particular variants having increased catalytic activity, specificity for the substrate, and /or stability.

In an embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous epi-cedrol synthase, i.e. an enzyme that catalyzes the formation of 8-epi-cedrol from farnesyl diphosphate or geranyl diphosphate. Examples of heterologous epi-cedrol synthases include, but are not limited to, epi-cedrol synthases from *Artemisia annua* (Genbank accession number: AAF80333, CAC08805). Any polypeptide exhibiting a epi-cedrol synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to any of these enzymes may be used, in particular variants having increased catalytic activity, specificity for the substrate, and /or stability.

In an embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous (3S, 6E)-nerolidol synthase, i.e. an enzyme that converts farnesyl diphosphate to nerolidol. Examples of heterologous nerolidol synthases include, but are not limited to, nerolidol synthases from *Selaginella moellendorffii* (Uniprot accession number D8RNZ9), *Populus trichocarpa* (F8TWD1), *Medicago truncatula* (Q5UB06), *Fragaria x ananassa* (P0CV94) and *Zea mays* (Degenhardt and Gershenzon, 2000). Any polypeptide exhibiting a nerolidol synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to any of these enzymes may be used, in particular variants of these enzymes having increased catalytic activity, specificity for the substrate, and /or stability. In a particular embodiment, the heterologous nerolidol synthase is selected from *Fragaria x ananassa*.

In an embodiment, the recombinant bacterium of the invention comprises a gene encoding a heterologous santalene synthase, i.e. an enzyme that catalyzes the formation of alpha-santalene, beta-santalene, epi-beta-santalene, endo-beta-bergamotene and/or exo-alpha-bergamotene from farnesyl diphosphate. Examples of heterologous santalene synthases include, but are not limited to, santalene synthase from *Solanum habrochaites* (Uniprot: B8XA41), *Santalum spicatum* (Uniprot: E3W202), *Santalum austrocaledonicum* (Uniprot: E3W203), *Santalum spicatum* (Uniprot: E3W204). Any polypeptide exhibiting a santalene synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to any of these enzymes may be used, in particular variants of these enzymes having increased catalytic activity, specificity for the substrate, and /or stability.

In an embodiment, the recombinant bacterium of the invention comprises a gene encoding a δ -cadinene synthase, i.e. an enzyme that catalyzes the cyclization of trans,trans-farnesyl diphosphate (FPP) to (+)-delta cadinene. Examples of heterologous δ -cadinene synthases include, but are not limited to, δ -cadinene synthases from
5 *Gossypium hirsutum* (GenBank accession number: AAF74977, AAC12784), *Gossypium arboreum* (GenBank accession number CAA65289, Uniprot Q39761) and *Helianthus annuus* (Q4U3F6). Any polypeptide exhibiting a δ -cadinene synthase activity and having at least 70%, 80%, preferably 90%, sequence identity to any of these enzymes may be used, in particular variants of these enzymes having increased catalytic activity,
10 specificity for the substrate, and /or stability. In a particular embodiment, the heterologous δ -cadinene synthase is selected from *Gossypium arboreum*.

Heterologous isoprene synthases

In another aspect, the present invention also relates to a recombinant *Deinococcus* bacterium comprising a gene encoding a heterologous isoprene synthase.

15 All embodiments described herein for the other aspects of the invention are also encompassed in this aspect. In particular, the recombinant *Deinococcus* bacterium may further exhibit (i) an enhanced MEP pathway as described above, optionally with an improved DXP synthase, (ii) a heterologous MEV pathway, (iii) an increase of the FPP synthase activity, (iv) inactivation of the lycopene beta-cyclase and/or (v) expression of
20 a heterologous terpene synthase as described herein.

In a preferred embodiment, the recombinant *Deinococcus* bacterium comprising a gene encoding a heterologous isoprene synthase, overexpresses at least one gene selected from the group consisting of *dxs*, *dxr*, *ispD*, *ispE*, *ispF* and *ispG*, preferably overexpresses the *dxs*, *dxr*, *ispD*, *ispE*, *ispF* and *ispG* genes.

25 The heterologous isoprene synthase may be any enzyme catalysing the formation of isoprene from dimethylallyl diphosphate. Examples of isoprene synthases include, but are not limited to, isoprene synthases from *Bacillus subtilis* (Hess et al. 2013), *Populus alba* (Lv et al. 2013; Yang et al. 2012; Uniprot: Q50L36), *Populus tremuloides* (Uniprot: Q7XAS7), *Pueraria montana* (Whited et al., 2010; Miller et al., 2001; Sharkey et al.,
30 2005) and *Quercus robur* (Lehning et al., 1999).

Farnesyl diphosphate synthase

In addition, the inventors herein demonstrated that the production of isoprenoids in *Deinococcus* bacteria can be controlled to promote the production and/or accumulation of compounds of interest. In this respect, they identified an enzyme of *Deinococcus*
5 *geothermalis* exhibiting a farnesyl diphosphate synthase activity (EC 2.5.1.10), dimethylallyltranstransferase activity (EC 2.5.1.1) and geranylgeranyl diphosphate synthase activity (EC 2.5.1.29) and observed that overexpression of this enzyme promotes the production of isoprenoids having more than 10 C-atoms, such as diterpenes, triterpenes, sesquiterpenes, and carotenoids, to the detriment of monoterpenes.

10 Thus, in a further aspect, the present invention relates to an isolated polypeptide having a farnesyl diphosphate synthase activity, which polypeptide is selected from the group consisting of

a) a polypeptide comprising all or an active part of the amino acid sequence of SEQ ID NO: 47;

15 b) a polypeptide having an amino acid sequence having at least 60, 65 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% sequence identity to SEQ ID NO: 47, preferably at least 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% sequence identity to SEQ ID NO: 47;

c) a polypeptide encoded by a nucleotide sequence having at least 60, 65, 70, 75,
20 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% sequence identity to SEQ ID NO: 46, preferably at least 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% sequence identity to SEQ ID NO: 46; or

d) a polypeptide which is encoded by a nucleic acid sequence which is capable of hybridizing under medium/high stringency, preferably high or very high, conditions with
25 (i) the nucleic acid sequence set forth in SEQ ID NO: 46, (ii) its complementary strand, or (iii) a subsequence of (i) or (ii).

Preferably, the polypeptide of the invention further exhibits dimethylallyltranstransferase (EC 2.5.1.1) and/or geranylgeranyl diphosphate synthase (EC 2.5.1.29) activities, more preferably dimethylallyltranstransferase and
30 geranylgeranyl diphosphate synthase activities.

Suitable experimental conditions for determining hybridization between a nucleotide probe and a homologous DNA or RNA sequence involves presoaking of the filter containing the DNA fragments or RNA to hybridize in 5×SSC (Sodium chloride/Sodium citrate for 10 min, and prehybridization of the filter in a solution of 5 5×SSC, 5×Denhardt's solution, 0.5% SDS and 100 µg/ml of denatured sonicated salmon sperm DNA, followed by hybridization in the same solution containing a concentration of 10 ng/ml of a random-primed ³²P-dCTP-labeled (specific activity >1×10⁹ cpm/µg) probe for 12 hours at ca. 45° C (Feinberg and Vogelstein, 1983). For various stringency conditions the filter is then washed twice for 30 minutes in 2×SSC, 0.5% SDS and at least 10 55° C. (low stringency), more preferably at least 60° C. (medium stringency), still more preferably at least 65° C. (medium/high stringency), even more preferably at least 70° C. (high stringency), and even more preferably at least 75° C. (very high stringency).

The present invention also relates to a nucleic acid encoding a polypeptide of the invention. The nucleic acid can be DNA (cDNA or gDNA), RNA, or a mixture of the 15 two. It can be in single stranded form or in duplex form or a mixture of the two. It can comprise modified nucleotides, comprising for example a modified bond, a modified purine or pyrimidine base, or a modified sugar. It can be prepared by any method known to one skilled in the art, including chemical synthesis, recombination, and mutagenesis. The nucleic acid according to the invention may be deduced from the sequence of the 20 polypeptide according to the invention and codon usage may be adapted according to the host cell in which the nucleic acid shall be transcribed. These steps may be carried out according to methods well known to one of skill in the art and some of which are described in the reference manual Sambrook *et al.* (Sambrook *et al.*, 2001).

The present invention further relates to an expression cassette comprising a 25 nucleic acid encoding a polypeptide according to the invention, operably linked to one or more control sequences that direct the expression of said nucleic acid in a suitable host cell under conditions compatible with the control sequences.

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 30 the enzyme. The promoter may be any polynucleotide that shows transcriptional activity in the *Deinococcus* bacterium, in particular a wild-type, mutated, degenerated or synthetic promoter. The promoter may be a native, homologous or heterologous promoter.

Preferred promoters are native or homologous. In this regard, 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, 5 the promoter region *PgroESL* of the *groESL* operon (Lecointe *et al*, 2004; Meima *et al*, 2001), or derivatives of such promoters.

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 10 be used in the present invention (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.

The present invention also relates to an expression vector comprising a nucleic 15 acid or an expression cassette according to the invention. Said expression vector may be used to transform a host cell, preferably *Deinococcus* host cells, and enable the expression of the nucleic acid of the invention in said cell. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. The vector may be an autonomously replicating vector, i.e., a vector that 20 exists as an extra-chromosomal entity, the replication of which is independent of chromosomal replication, e.g., a plasmid, an extra-chromosomal element, a mini-chromosome, or an artificial chromosome. The vector may contain any means for assuring self-replication. Alternatively, the vector may be one that, when introduced into the host cell, is integrated into the genome and replicated together with the 25 chromosome(s) into which it has been integrated.

The vector preferably comprises one or more selectable markers that permit easy selection of host cells comprising the vector. A selectable marker is a gene the product of which provides for biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophy, and the like.

30 The vector preferably comprises an element that permits integration of the vector into the host cell's genome or autonomous replication of the vector in the cell independent

of the genome. When integration into the host cell genome occurs, integration of the sequences into the genome may rely on homologous or non-homologous recombination. In one hand, the vector may contain additional polynucleotides for directing integration by homologous recombination at a precise location into the genome of the host cell. These
5 additional polynucleotides may be any sequence that is homologous with the target sequence in the genome of the host cell. On the other hand, the vector may be integrated into the genome of the host cell by non-homologous recombination.

For autonomous replication, the vector may further comprise an origin of replication enabling the vector to replicate autonomously in the host cell in question. The
10 origin of replication may be any plasmid replicator mediating autonomous replication that functions in a cell. The term "origin of replication" or "plasmid replicator" means a polynucleotide that enables a plasmid or vector to replicate *in vivo*.

The methods for selecting these elements according to the host cell in which expression is desired, are well known to one of skill in the art. The vectors may be
15 constructed by the classical techniques of molecular biology, well known to one of skill in the art.

The present invention further relates to the use of a nucleic acid, an expression cassette or an expression vector according to the invention to transform, transfect or transduce a cell, preferably a *Deinococcus* bacterium. The present invention also relates
20 to a host cell, preferably a *Deinococcus* bacterium, comprising a nucleic acid, a cassette or an expression vector according to the invention.

The host cell may be transformed, transfected or transduced in a transient or stable manner. An expression cassette or vector of the invention is introduced into a host cell so that the cassette or vector is maintained as a chromosomal integrant or as a self-replicating
25 extra-chromosomal vector as described earlier. The term "host cell" also encompasses any progeny of a parent host cell that is not identical to the parent host cell due to mutations that occur during replication.

The nucleic acid, expression cassette or expression vector according to the invention may be introduced into the host cell by any method known by the skilled person,
30 such as electroporation, conjugation, transduction, competent cell transformation, protoplast transformation, protoplast fusion, biolistic "gene gun" transformation, PEG-

mediated transformation, lipid-assisted transformation or transfection, chemically mediated transfection, lithium acetate-mediated transformation, liposome-mediated transformation,

Optionally, more than one copy of a nucleic acid, cassette or vector of the present
5 invention may be inserted into the host cell.

As mentioned above, the inventors observed that overexpression of the FPP synthase promotes the production of isoprenoids having more than 10 C-atoms, such as diterpenes, triterpenes, sesquiterpenes, and carotenoids, to the detriment of monoterpenes.

Thus, the present invention thus also relates to a recombinant *Deinococcus*
10 bacterium wherein the FPP synthase activity is increased. Preferably, the FPP synthase activity is increased by overexpression of a native, homologous or heterologous gene encoding a FPP synthase, i.e. a *ispA* gene. Gene overexpression may be obtained as described above.

The overexpressed FPP synthase may be any heterologous FPP synthase.
15 Examples of heterologous FPP synthases include, but are not limited to, FPP synthases from *Saccharomyces cerevisiae* (Uniprot Q12051) or from *E. coli* (Uniprot P22939). In a particular embodiment, the recombinant bacterium of the invention expresses the FPP synthase from *E. coli*.

In a preferred embodiment, the overexpressed FPP synthase is from a
20 *Deinococcus* bacterium. In particular, the overexpressed FPP synthase is a polypeptide of the invention as described above and having a farnesyl diphosphate synthase activity, preferably farnesyl diphosphate synthase, dimethylallyltranstransferase and geranylgeranyl diphosphate synthase activities. Preferably, the overexpressed FPP synthase is a FPP synthase from *D. geothermalis*, in particular a FPP synthase having all
25 or part of the amino acid sequence set forth in SEQ ID NO: 47.

Alternatively, the farnesyl diphosphate synthase, dimethylallyltranstransferase and geranylgeranyl diphosphate synthase activities may be carried out by separate polypeptides and the increased activities may result from the overexpression of separate encoding genes.

The inventors also showed that the substitution K170G in the FPP synthase set forth in SEQ ID NO: 47 induced a redirection of the carbon flux toward GPP and thus toward production of monoterpenes. Indeed, the K170G mutation in the FPPS allows GPP release for monoterpene biosynthesis (Blanchard and Karst, 1993; Fischer et.al, 2011).
5 Thus, when production of a monoterpene such as geraniol is desired, the endogenous FPP synthase may be replaced by the K170G mutant. In the present document, the following terminology is used to designate a substitution: K170G denotes that amino acid residue at position 170 of SEQ ID NO: 47 (lysine, K) is changed to a glycine (G).

Thus, the present invention also relates to a polypeptide of the invention wherein
10 the lysine residue at position corresponding to position 170 in SEQ ID NO: 47 is substituted with a glycine (the corresponding position can be easily identified by the skilled person using well-known alignment algorithms), a nucleic acid encoding said polypeptide, an expression cassette or vector comprising said nucleic acid and a recombinant *Deinococcus* bacterium expressing said mutated FPP synthase. Preferably,
15 said recombinant bacterium also expresses a heterologous terpene synthase, preferably a monoterpene synthase. This recombinant is preferably used to produce monoterpenes.

All embodiments described herein for the other aspects of the invention are also encompassed in this aspect. In particular, the recombinant *Deinococcus* bacterium may further exhibit (i) an enhanced MEP pathway as described above, optionally with an
20 improved DXP synthase, (ii) a heterologous MEV pathway, (iii) inactivation of the lycopene beta-cyclase and/or (iv) expression of a heterologous terpene or isoprene synthase as described herein.

Inactivation of the endogenous lycopene beta-cyclase

The inventors also showed that the inactivation of the endogenous lycopene beta-
25 cyclase induces an overproduction/accumulation of lycopene.

Thus, in another aspect, the present invention relates to a recombinant *Deinococcus* bacterium wherein the endogenous lycopene beta-cyclase activity is reduced or suppressed.

The lycopene beta-cyclase (EC 5.5.1.19) catalyzes the reaction from lycopene to
30 beta-carotene via the intermediate gamma-carotene. Examples of lycopene beta-cyclases

include, but are not limited to, lycopene beta-cyclases of *D. geothermalis* having the amino acid sequence set forth in SEQ ID NO 51, *D. phoenicis* (Genbank accession number: EYB66908), *D. apachensis* (WP_019585057), *D. aquatilis* (WP_019008078), *D. gobiensis* (WP_014685779), *D. wulumuqiensis* (WP_017870547), *D. maricopensis* 5 (WP_013556733) and *D. radiodurans* (WP_010887447).

The gene encoding the lycopene beta-cyclase may also be easily identified based on homology with the nucleic acid encoding the lycopene beta-cyclase of *D. geothermalis* (SEQ ID NO: 50).

The lycopene beta-cyclase activity may be reduced or suppressed using any 10 method known by the skilled person. Preferably, the gene encoding the lycopene beta-cyclase is 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, a cassette, a gene or a mutation inducing a frameshift. In a preferred embodiment, the gene encoding the lycopene beta-cyclase is deleted.

15 In a particular embodiment, genes encoding phytoene synthase (EC 2.5.1.32) and phytoene desaturase (EC 1.3.99.28) are overexpressed in order to increase lycopene production. Genes may be native, homologous or heterologous genes, preferably native or homologous genes.

In another particular embodiment, the recombinant bacterium having reduced or 20 suppressed lycopene beta-cyclase activity, and optionally overexpressing genes encoding phytoene synthase and phytoene desaturase, further comprises a gene encoding a heterologous carotenoid cleavage dioxygenase (CCD) catalysing the formation of apocarotenoids from lycopene.

In another particular embodiment, the recombinant bacterium having reduced or 25 suppressed lycopene beta-cyclase activity, and optionally overexpressing genes encoding phytoene synthase and phytoene desaturase, further comprises a gene encoding a heterologous lycopene epsilon cyclase (EC 5.5.1.18) catalysing the formation of δ -carotene from lycopene and optionally a gene encoding a heterologous carotenoid cleavage dioxygenase (CCD) catalysing the formation of apocarotenoids from δ -carotene.

Examples of carotenoid cleavage dioxygenases include, but are not limited to, CCD disclosed in the article of Auldridge et al. (2006) such as CCD from *Arabidopsis thaliana*, *Rubus idaeus* and *Zea mais*.

All embodiments described herein for the other aspects of the invention are also
5 encompassed in this aspect. In particular, the recombinant *Deinococcus* bacterium may further exhibit (i) an increase of the FPP synthase activity and (ii) an enhanced MEP pathway as described above, optionally with an improved DXP synthase as described above, (iii) a heterologous MEV pathway and/or (iv) expression of a heterologous terpene or isoprene synthase as described above.

10 **Method of producing an isoprenoid compound**

In a further aspect, the present invention relates to a use of a recombinant *Deinococcus* bacterium as described above for producing a terpene or terpenoid compound. The present invention also relates to a method of producing a terpene or terpenoid comprising (i) culturing a recombinant *Deinococcus* bacterium according to the
15 invention under conditions suitable to produce the terpene or terpenoid and optionally (ii) recovering the terpene or terpenoid.

The terpene or terpenoid compound may be any terpene or terpenoid compound produced by a recombinant *Deinococcus* bacterium as described above.

In an embodiment, the recombinant *Deinococcus* bacterium of the invention is
20 used to produce isoprenoids having more than 10 C-atoms, such as carotenoid compounds (C40). In particular, the present invention relates to a method of producing a carotenoid compound comprising (i) culturing a recombinant *Deinococcus* bacterium according to the invention under conditions suitable to produce the carotenoid compound and optionally (ii) recovering the carotenoid compound. The carotenoid compound may
25 be lycopene or any other carotenoid compound derived from lycopene, preferably deinoxanthine. In a particular embodiment, the recombinant *Deinococcus* bacterium overexpresses FPP synthase, preferably *Deinococcus* FPP synthase and more preferably a FPP synthase of the invention as described above.

In an embodiment, the recombinant *Deinococcus* bacterium comprises a
30 heterologous terpene synthase as described above and may produce a terpene or terpenoid

compound selected from the group consisting of geraniol, (+)-linalool, (-)-linalool, S-limonene, R-limonene, α -pinene, β -pinene, fenchol, terpineol, (-)-carvone, (+)-(S)-carvone, menthone, isomenthone, menthol, noementhol, (S,E)-nerolidol, 8-epi-cedrol, bisabolol, germacrene A, germacrene B, germacrene C, germacrene D, valencene, 5 patchoulol, santalene and δ -cadinene.

The method may further comprise converting the terpene or terpenoid into a terpene derivative, such as myrcenol and dihydromyrcenyl acetate obtained from myrcene; fenchyl and borneol derivatives obtained from fenchol; nootkatone obtained from valencene or (-)-beta-pinene; linalyl and lavandulyl esters obtained from linalool 10 such as linalyl formate, linalyl acetate, linalyl propionate, linalyl butyrate, linalyl isobutyrate and lavandulyl acetate; citronellyl and dihydromyrcenyl esters obtained from citronellol such as citronellyl formate, citronellyl acetate, citronellyl propionate, citronellyl isobutyrate, citronellyl isovalerate, citronellyl tiglate, citronellic acid nitrile (or citronellyl nitrile); geranyl esters obtained from geraniol such as geranyl formate, geranyl 15 acetate, geranyl propionate, geranyl isobutyrate, neryl acetate, methyl geranate (or geranic acid methyl ester) and geranic acid nitrile (or geranonitrile). Preferably, the terpene derivative is a terpene ester, more preferably is a geranyl ester.

Methods for obtaining terpene derivatives from terpenes or terpenoids are well known by the skilled person. For example, such methods are disclosed in Common 20 Fragrance and Flavor Materials: Preparation, Properties and Uses, 5th ed. H. Surburg and J. Panten, John Wiley & Sons, 2006.

Conditions suitable to produce the terpene or terpenoid may be easily determined by the skilled person according to the *Deinococcus* bacterium used in the present invention. In particular, the carbon source may be selected from the group consisting of 25 C5 sugars such as xylose and arabinose, C6 sugars such as glucose, cellobiose, saccharose and starch. When the *Deinococcus* bacterium exhibits cellulolytic or xylanolytic activity, more complex carbon sources can be used such as cellulosic or lignocellulosic biomass. Temperature conditions can also be adapted depending on the use of mesophilic or thermophilic *Deinococcus* bacteria.

In another aspect, the present invention also relates to a method of producing a terpene derivative, in particular a terpene derivative as described above, comprising (i) culturing a recombinant *Deinococcus* bacterium according to the invention under conditions suitable to produce a terpene or terpenoid and optionally recovering said
5 terpene or terpenoid, and (iii) converting said terpene or terpenoid into a terpene derivative, and optionally (iv) recovering the terpene derivative. Step (iii) may be a chemical or enzymatic conversion.

Preferably, the terpene or terpenoid is geraniol and/or geranic acid and the terpene derivative is a geranyl ester.

10

The inventors found that non-modified *Deinococcus* bacteria are able to convert geraniol to geranial and geranic acid. Thus, in a particular aspect, the present invention relates to the use of a recombinant *Deinococcus* bacterium of the invention for producing geraniol, geranial and/or geranic acid. The present invention also relates to a method of
15 producing geraniol, geranial and/or geranic acid comprising (i) culturing a recombinant *Deinococcus* bacterium according to the invention under conditions suitable to produce geraniol, geranial and/or geranic acid and optionally (ii) recovering geraniol, geranial and/or geranic acid.

Preferably, the recombinant *Deinococcus* bacteria comprises a gene encoding a
20 heterologous geraniol synthase, and optionally exhibits an enhanced MEP pathway, optionally with an improved DXP synthase and/or a heterologous MEV pathway as disclosed above.

In another aspect, the present invention also relates to a method of producing
25 geranial and/or geranic acid comprising (i) contacting a *Deinococcus* bacterium, preferably a recombinant *Deinococcus* bacterium of the invention, or an extract thereof, with geraniol, and optionally (ii) recovering geranial and/or geranic acid.

In another particular aspect, the present invention relates to a use of a recombinant *Deinococcus* bacterium of the invention for producing a geranyl ester. The present invention also relates to a method of producing a geranyl ester comprising (i) culturing a recombinant *Deinococcus* bacterium according to the invention under conditions suitable
5 to produce geraniol, geranial and/or geranic acid, optionally (ii) recovering geraniol, geranial and/or geranic acid, and (iii) converting geraniol, geranial and/or geranic acid into a geranyl ester, and optionally (iv) recovering the geranyl ester. Step (iii) may be a chemical or enzymatic conversion.

Preferably, the recombinant *Deinococcus* bacteria comprises a gene encoding a
10 heterologous geraniol synthase, an optionally exhibits an enhanced MEP pathway, optionally with an improved DXP synthase and/or a heterologous MEV pathway as disclosed above.

Preferably, the geranyl ester is methyl geranate, geranyl formate, geranyl acetate, geranyl propionate, geranyl isobutyrate, *trans*-geranyl acetate or neryl acetate. These
15 esters may be produced from geraniol, geranial and/or geranic acid by any method known by the skilled person.

In another aspect, the present invention relates to a use of a recombinant *Deinococcus* bacterium of the invention for producing an apocarotenoid. Preferably, the
20 apocarotenoid is selected from the group consisting of damascones, damascenones and ionones. Examples of damascone, damascenone and ionone include, but are not limited to, β -damascenone, *trans*- β -damascenone, γ -damascone, α -damascone, *trans*- α -damascone, *cis*- α -damascone, *cis*-beta-damascone, *trans*-beta-damascone, delta-damascone, *trans,trans*-delta-damascone, *trans*-(2,4,4-trimethyl-2-cyclohexenyl)-2-
25 butenone, 1-(2,4,4-trimethyl-2-cyclohexen-1-yl)-2-buten-1-one, 1-(2,4,4-trimethyl-1-cyclohexenyl)-2-butenone, 1-(2,2-dimethyl-6-methylenecyclohexyl)-2-buten-1-one, alpha-ionone, beta-ionone and pseudo-ionone.

Thus, the present invention relates to a method of producing an apocarotenoid, i.e. damascone, damascenone and/or ionone, comprising (i) culturing a recombinant
30 *Deinococcus* bacterium according to the invention under conditions suitable to produce geraniol, geranial and/or geranic acid, optionally (ii) recovering geraniol, geranial and/or

geranic acid, and (iii) converting geraniol, geranial and/or geranic acid into said apocarotenoid, and optionally (iv) recovering said apocarotenoid.

Conversion of geraniol, geranial and/or geranic acid into the damascone, damascenone and/or ionone may involve an intermediate conversion of geraniol, geranial
5 and/or geranic acid into a geranyl ester, preferably methyl geranate.

Preferably, the recombinant *Deinococcus* bacteria comprises a gene encoding a heterologous geraniol synthase, an optionally exhibits an enhanced MEP pathway, optionally with an improved DXP synthase and/or a heterologous MEV pathway as disclosed above. Methods for converting geraniol, geranial and/or geranic acid into the
10 damascone, damascenone and/or ionone are well-known by the skilled person.

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

Preferably, the recombinant *Deinococcus* bacterium is a recombinant as described above wherein the gene encoding the lycopene beta-cyclase is inactivated, and further comprising a gene encoding a heterologous carotenoid cleavage dioxygenase (CCD). This recombinant may further comprise a gene encoding a heterologous lycopene epsilon
20 cyclase. Optionally, genes encoding phytoene synthase and phytoene desaturase may also be overexpressed. The recombinant *Deinococcus* bacterium may further exhibit an enhanced MEP pathway, optionally with an improved DXP synthase and/or a heterologous MEV pathway and/or overexpression of FPP synthase as disclosed above.

25 The present invention also relates to a method of producing an apocarotenoid, i.e. damascone, damascenone and/or ionone, comprising (i) culturing a recombinant *Deinococcus* bacterium according to the invention under conditions suitable to produce β -carotene, (ii) recovering β -carotene, and (iii) converting β -carotene into said apocarotenoid, and optionally (iv) recovering said apocarotenoid.

Conversion of β -carotene into said apocarotenoid, such as damascone, damascenone, pseudoionones and/or ionone, may be chemical or enzymatic conversion and is well known by the skilled person.

Preferably, the recombinant *Deinococcus* bacterium is a recombinant as described
5 above exhibiting an enhanced MEP pathway, optionally with an improved DXP synthase and/or a heterologous MEV pathway and/or overexpression of FPP synthase. Optionally, genes encoding phytoene synthase and phytoene desaturase may also be overexpressed.

The present invention also relates to a method of producing an apocarotenoid
10 comprising (i) culturing a recombinant *Deinococcus* bacterium according to claim 13 under conditions suitable to produce lycopene, α -, β -, and/or γ -carotene, (ii) optionally recovering lycopene, α -, β -, and/or γ -carotene, and (iii) chemically or enzymatically converting lycopene, α -, β -, and/or γ -carotene into a apocarotenoid, and optionally (iv) recovering the apocarotenoid

15 Preferably, the recombinant *Deinococcus* bacterium is a recombinant as described above, in particular a recombinant comprising a gene encoding a heterologous CDD. The recombinant may further exhibit an enhanced MEP pathway, optionally with an improved DXP synthase and/or a heterologous MEV pathway and/or overexpression of FPP synthase. Optionally, genes encoding phytoene synthase and phytoene desaturase may
20 also be overexpressed.

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

25 **Example 1: Production of geraniol and geranic acid**

A *Deinococcus geothermalis* strain was genetically engineered to produce geraniol and geranic acid. A heterologous gene encoding a geraniol synthase (GES) from *Ocimum basilicum* was inserted into chromosome replacing phosphotransacetylase (pta) gene. Expression of GES gene was under the control of a strong constitutive promoter.

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 ; NH_4Cl 20 mM ; NaOH 10 mM ; KOH 10 mM ; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0,5 μM ; $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ 0.276 mM ; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 0.528 mM ; $(\text{NH}_4)_6(\text{Mo}_7)\text{O}_{24} \cdot 4\text{H}_2\text{O}$ 3 nM ; 5 H_3BO_3 0.4 μM ; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 30 nM ; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 10 nM ; MnCl_2 0.25 μM ; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 10 nM ; D-Biotin 1 $\mu\text{g/L}$; Niacin (nicotinic acid) 1 $\mu\text{g/L}$; B6 vitamin 1 $\mu\text{g/L}$; B1 vitamin ; FeCl_3 20 μM ; Sodium Citrate $\cdot 2\text{H}_2\text{O}$ 20 μM ; K_2HPO_4 5,7 mM) containing 2% 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 25 ml of the same 10 fresh medium at an initial optical density at 600 nm (OD600) of 0.4. This second seed culture was cultured at 37°C and 250 rpm overnight.

The cultures for geraniol production were performed at 37°C and 250 rpm for 48 h from log phase of growth inoculated into 25 ml of mineral define medium ($(\text{NH}_4)_2\text{SO}_4$ < 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 15 < 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 density at 600 nm (OD600) of 0.4.

After 24H of culture, geraniol was produced (titer: 2.01 mg/L ; 1.5 mg/g DCW 20 (dry cell weight) ; 0.6 mg/g of glucose) (Figure 1). After 48H of culture geraniol was produced (titer: 3.9 mg/L ; 0.5 mg/g DCW; 0.3 mg/g of glucose) and geranic acid was detected (Figure 2). After 72H of culture, a few geraniol remains and a major peak of geranic acid was present (Figure 3). The extractions were done by mixing 0.5mL of ethyl acetate with 1 mL of culture and separated by centrifugation. The ethyl acetate phase was 25 analyzed by GC–mass spectrometry (GC–MS).

Therefore, the production of geraniol was identified and quantified on a Mass Spectrometer GC. Gas chromatography was performed using GC-MS [Agilent 7890A/5975, column: 0.25 mm $\times$ 30 m, 0.25 μm (HP-5MS ultra inert)] fitted with a mass spectrometer as detector. GCMS analyse was conducted under electron-impact (EI) mode 30 (70 eV). 1 μL of extract was injected in pulsed splitless mode (50 psi for until 0.75min). The scan range covered 50–250 m/z. The data were processed using Xcalibur system.

Identification were performed using NIST library. The standard curves of geraniol (Figure 4) were constructed using the same method described as above.

The production of geranic acid was identified and quantified on high-pressure liquid chromatography. HPLC was performed using an Agilent C18 poroshell column 5 (EC-C18) 4.6*150mm-2.7um and a mobile phase Acetonitrile/water (0.1% formic acid). 10 µL of extract was injected. The standard curves of geranic acid (Figure 5) were constructed using the same method described as above.

Example 2: Production of lycopene

A *Deinococcus geothermalis* strain was genetically engineered to produce lycopene. The wild-type bacterium comprises related genes for synthesizing the lycopene through a MEP pathway. The recombinant *D. geothermalis* producing lycopene was obtained by disrupting a part of the carotenoid pathway, i.e. the lycopene beta-cyclase (EC 5.5.1.19) (*crtlm*) gene was knockout. The resulting constructs were checked by sequencing.

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 2% 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 25 ml of the same fresh medium at an initial optical density at 600 nm (OD₆₀₀) of 0.4. This second seed culture was cultured at 37°C or 45°C and 250 rpm overnight.

The cultures for lycopene production were performed at 37°C or 45°C and 250 rpm for 48 h from log phase of growth inoculated into 25 ml of mineral define medium (NH₄)₂SO₄ < 100 mM ; NaH₂PO₄.H₂O < 10 mM ; KCl < 10 mM ; Na₂SO₄ < 10 mM ; Acide citrique < 30 mM ; MgCl₂.6H₂O < 10 mM ; 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 ; 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 density at 600 nm (OD600) of 10.

After 24h of culture, the lycopene producing strain was at OD600 of 31. The lycopene was produced with a titer of 4.2 mg/L, a yield of 0.5 mg/g DCW (dry cell weight) and 0.3 mg/g of glucose. 1 mL of culture was centrifuged and lycopene extraction was done by mixing 0.9 mL of acetone with pellet. The acetone phase was analyzed by absorbance at OD 472 nm (figure 6). Finally the lycopene concentration was determined by the dilution of lycopene standard.

Example 3 : Overexpression of the FPP synthase

10 The farnesyl pyrophosphate synthase (FPPS) gene from *Deinococcus geothermalis* (SEQ ID NO: 46) was inserted in the genome of the recombinant *D. geothermalis* expressing the GES of *Ocimum basilicum* of example 1 and the recombinant *D. geothermalis* producing lycopene of example 2, under the control of a strong constitutive promoter. The resulting constructs were checked by sequencing.

15 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 ; NH_4Cl 20 mM ; NaOH 10 mM ; KOH 10 mM ; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0,5 μM ; $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ 0.276 mM ; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 0.528 mM ; $(\text{NH}_4)_6(\text{Mo}_7)\text{O}_{24} \cdot 4\text{H}_2\text{O}$ 3 nM ; H_3BO_3 0.4 μM ; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 30 nM ; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 10 nM ; MnCl_2 0.25 μM ;
20 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 10 nM ; D-Biotin 1 $\mu\text{g/L}$; Niacin (nicotinic acid) 1 $\mu\text{g/L}$; B6 vitamin 1 $\mu\text{g/L}$; B1 vitamin ; FeCl_3 20 μM ; Sodium Citrate $\cdot 2\text{H}_2\text{O}$ 20 μM ; K_2HPO_4 5,7 mM) containing 2% 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 25 ml of the same fresh medium at an initial optical density at 600 nm (OD600) of 0.4. This second seed
25 culture was cultured at 37°C and 250 rpm overnight.

The cultures for geraniol production were performed at 37°C or 45°C and 250 rpm for 24H and 48 h from log phase of growth inoculated into 25 ml of mineral define medium $(\text{NH}_4)_2\text{SO}_4$ < 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
30 < 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 ;

$\text{CoCl}_2 \cdot 6\text{H}_2\text{O} < 50 \text{ mg/L}$; $\text{H}_3\text{BO}_3 < 5 \text{ mg/L}$; $\text{MES} < 200 \text{ mM}$; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O} < 0,5 \text{ mM}$; Glucose $< 30 \text{ g/L}$ (166 mM) at an initial optical density at 600 nm (OD600) of 0.4.

The cultures for lycopene production were performed following the same protocole as described above. The differences being the initial optical density at 600 nm 5 (OD600) of 10.

The inventors observed that overexpression of FPP synthase resulted in a significant increase in lycopene production (Figure 7). On the contrary, the geraniol production was dramatically reduced (Figure 8).

Example 4 : Overexpression of MEP enzymes

10 The 1-Deoxy-D-xylulose 5-phosphate synthase (DXS) gene (SEQ ID NO: 3), the 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) gene (SEQ ID NO: 36), the 2-C-methyl-D-erythritol-4-phosphate cytidyltransferase (IspD) gene (SEQ ID NO: 19), the 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase (IspE) gene (SEQ ID NO: 40), the 2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase (IspF) gene (SEQ ID NO: 23),
15 (E)-4-hydroxy-3-methylbut-2-enyl-diphosphate synthase (IspG) gene (SEQ ID NO: 27), the 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (IspH) gene (SEQ ID NO: 44) or the 4-hydroxy-3-methylbut-2-enyl-diphosphatereductase (IDI) gene (SEQ ID NO: 31) from *Deinococcus yunweiensis*, was inserted into the chromosome of the recombinant *D. geothermalis* bacterium expressing the GES of *Ocimum basilicum* as described in
20 example 1, under the control of a strong constitutive promoter. The resulting constructs were checked by sequencing.

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 ; NH_4Cl 20 mM ; NaOH 10 mM ; KOH 10 mM ; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0,5 μM ;
25 $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ 0.276 mM ; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 0.528 mM ; $(\text{NH}_4)_6(\text{Mo}_7)\text{O}_{24} \cdot 4\text{H}_2\text{O}$ 3 nM ; H_3BO_3 0.4 μM ; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 30 nM ; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 10 nM ; MnCl_2 0.25 μM ; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 10 nM ; D-Biotin 1 $\mu\text{g/L}$; Niacin (nicotinic acid) 1 $\mu\text{g/L}$; B6 vitamin 1 $\mu\text{g/L}$; B1 vitamin ; FeCl_3 20 μM ; Sodium Citrate $\cdot 2\text{H}_2\text{O}$ 20 μM ; K_2HPO_4 5,7 mM) containing 2% glucose as the main carbon source, and cultured at 37°C and 250 rpm
30 overnight. Seed from log phase of growth was then inoculated into 25 ml of the same

fresh medium at an initial optical density at 600 nm (OD600) of 0.4. This second seed culture was cultured at 37°C and 250 rpm overnight.

The cultures for geraniol production were performed at 37°C and 250 rpm for 48 h from log phase of growth inoculated into 25 ml of mineral define medium (NH₄)₂SO₄ 5 < 100 mM ; NaH₂PO₄.H₂O < 10 mM ; KCl < 10 mM ; Na₂SO₄ < 10 mM ; Acide citrique < 30 mM ; MgCl₂.6H₂O < 10 mM ; 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.

10 The inventors observed that overexpression of at least one MEP enzyme resulted in a significant increase in geraniol production used herein as marker for isoprenoid production (Figure 9).

Example 5 : Improved DXS enzyme from *Deinococcus yunweiensis*

The 1-Deoxy-D-xylulose 5-phosphate synthase (DXS) gene from *Deinococcus*
15 *yunweiensis* (SEQ ID NO:3) was inserted into the chromosome, replacing the endogenous *dxs* gene, of the recombinant *D. geothermalis* bacterium expressing the GES of *Ocimum basilicum* as described in example 1, under the control of a strong constitutive promoter. Site directed mutagenesis was realized to obtain R238C mutant (SEQ ID NO: 13 and 14). The resulting constructs were checked by sequencing.

20 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 ;
25 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 2% 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 25 ml of the same fresh medium at an initial optical density at 600 nm (OD600) of 0.4. This second seed
30 culture was cultured at 37°C and 250 rpm overnight.

The cultures for geraniol production were performed at 37°C and 250 rpm for 48 h from log phase of growth inoculated into 25 ml of mineral define medium (NH₄)₂SO₄ < 100 mM ; NaH₂PO₄.H₂O < 10 mM ; KCl < 10 mM ; Na₂SO₄ < 10 mM ; Acide citrique < 30 mM ; MgCl₂.6H₂O < 10 mM ; CaCl₂.2H₂O < 10 mM ; ZnCl₂ < 50 mg/L ; 5 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.

As shown in Figure 10, improvement of the DXS by introduction of the mutation R238C results in a dramatic increase of geraniol production used herein as marker for 10 isoprenoid production.

Example 6 : Improved DXS enzyme from *Deinococcus radiopugnans*

A gene encoding 1-Deoxy-D-xylulose 5-phosphate synthase (DXS) from *Deinococcus radiopugnans* (SEQ ID NO: 52) or encoding R244C mutant of said enzyme (SEQ ID NO: 8) was inserted into the chromosome, replacing the endogenous *dxs* gene, 15 of the recombinant *D. geothermalis* bacterium expressing the GES of *Ocimum basilicum* as described in example 1, under the control of a strong constitutive promoter. The resulting constructs were checked by sequencing. Seed cultures and cultures for geraniol production were performed as detailed in example 5.

As shown in Figure 11, improvement of the DXS from *Deinococcus radiopugnans* 20 by introduction of the mutation R244C results in a dramatic increase of geraniol production used herein as marker for isoprenoid production.

Example 7 : K170G mutant of *D. geothermalis* farnesyl pyrophosphate synthase

The *D. geothermalis* farnesyl pyrophosphate synthase (FPPS) gene or the gene 25 encoding the K170G mutant of said enzyme obtained by site directed mutagenesis, was inserted in the recombinant *D. geothermalis* bacterium expressing the GES of *Ocimum basilicum* as described in example 1, into chromosome replacing the endogenous gene encoding FPPS. Both wild-type and mutated version of *D. geothermalis* FPPS gene was under the control of a constitutive promoter. The resulting constructs were checked by

sequencing. Seed cultures and cultures for geraniol production were performed as detailed in example 5.

As shown in Figure 12, the mutation K170G of *D. geothermalis* FPPS resulted in a redirection of the carbon flux through GPP and a significant increase in geraniol 5 production.

Example 8 : Overexpression of MEP enzymes in combination with improved DXS enzyme

The gene encoding the R238C mutant of DXS enzyme of *Deinococcus yunweiniensis* was inserted into chromosome replacing amylase (*amy*) gene of the 10 recombinant *D. geothermalis* expressing the GES of *Ocimum basilicum* of example 1, under the control of a constitutive promoter. The gene encoding the K170G mutant of *D. geothermalis* farnesyl pyrophosphate synthase (FPPS) gene was also inserted into chromosome replacing the endogenous *ispA* gene. Overexpression of FDPS gene was under the control of a constitutive promoter. The resulting constructs were checked by 15 sequencing.

Each of the following MEP pathways genes from *Deinococcus yunweiniensis* was expressed under the control of a strong constitutive promoter in the *D. geothermalis* strain expressing *D. geothermalis* FDPS (K170G mutant) and *D. yunweiniensis* DXS (R238C mutant) : *dxr* gene (SEQ ID NO : 36), *ispD* gene (SEQ ID NO : 19), *ispE* gene (SEQ ID 20 NO : 40), *ispF* gene (SEQ ID NO : 23), *ispG* gene (SEQ ID NO : 27) and *idi* gene (SEQ ID NO : 31). The resulting constructs were checked by sequencing. Seed cultures and cultures for geraniol production were performed as detailed in example 5.

As shown in Figure 13, overexpression of mutated DXS and IDI resulted in a dramatic increase in geraniol production by comparison with the other combinations.

25 Example 9 : Production of bisabolol

A *Deinococcus geothermalis* strain was genetically engineered to produce bisabolol. A heterologous gene encoding a (+)-epi-alpha-bisabolol synthase from *Streptomyces citricolor* was inserted into chromosome replacing phosphotransacetylase

(pta) gene. Expression of epi-alpha-bisabolol synthase gene was under the control of a constitutive promoter.

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 5 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 2% 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 25 ml of the same fresh medium at an initial optical density at 600 nm (OD₆₀₀) of 0.4. This second seed culture was cultured at 37°C and 250 rpm overnight. The cultures for bisabolol production were performed at 37°C and 250 rpm for 48 h from log phase of growth inoculated into 25 ml of mineral define medium (NH₄)₂SO₄ < 100 mM ; NaH₂PO₄.H₂O < 10 mM ; KCl < 10 mM ; Na₂SO₄ < 10 mM ; Acide citrique < 30 mM ; MgCl₂.6H₂O < 10 mM ; 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 production of bisabolol was identified and quantified on a Mass Spectrometer GC as detailed in example 1 for geraniol.

As shown in Figure 14, the expression of bisabolol synthase from *Streptomyces citricolor* in *D. geothermalis* resulted in a bisabolol production (3.6 mg/L).

25 Example 10 : Improved production of lycopene

A *Deinococcus geothermalis* strain was genetically engineered to produce lycopene as previously described in example 2. The gene encoding the R238C mutant of DXS enzyme of *D. yunweiniensis* and the gene encoding IDI of *D. yunweiniensis* were introduced into chromosome of the strain *D. geothermalis crtIm-*, under the control of a

constitutive promoter. Seed cultures and cultures for lycopene production were performed as detailed in example 2.

As shown in Figure 15, the lycopene production is two-fold higher when the strain *D. geothermalis crtItm*- overexpresses IDI and mutant DXS.

5 **Example 11 : Expression in *D. geothermalis* of GES of different origins**

A *Deinococcus geothermalis* strain was genetically engineered as described in example 1 by inserting a heterologous gene encoding a geraniol synthase (GES) from *Phyla dulcis* or *Cicer arietinum* into chromosome replacing phosphotransacetylase (pta) gene. Expression of GES gene was under the control of a strong constitutive promoter.

10 The resulting constructs were checked by sequencing. Seed cultures and cultures for geraniol production were performed as detailed in example 1.

As shown in Figure 16, the expression of GES of *Phyla dulcis* or *Cicer arietinum* also resulted in production of geraniol.

Example 12 : Carotenoid production

15 An expression cassette comprising the farnesyl pyrophosphate synthase (FPPS) gene from *Deinococcus geothermalis* (SEQ ID NO: 46) under the control of a strong promoter was inserted in the genome of *D. geothermalis* strain. The resulting constructs were checked by sequencing.

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 2% glucose as the main carbon source, and cultured at 45°C and 250 rpm overnight. Seed from log phase of growth was then inoculated into 25 ml of the same fresh medium at an initial optical density at 600 nm (OD₆₀₀) of 0.4. This second seed culture was cultured at 45°C and 250 rpm overnight.

The cultures for carotenoid production were performed at 45°C and 250 rpm for 48 h from log phase of growth inoculated into 25 ml of mineral define medium (NH₄)₂SO₄ < 100 mM ; NaH₂PO₄.H₂O < 10 mM ; KCl < 10 mM ; Na₂SO₄ < 10 mM ; Acide citrique < 30 mM ; MgCl₂.6H₂O < 10 mM ; 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.

25 mL of culture was centrifuged and carotenoid extraction was done by mixing 25 mL of ethanol with pellet. Spectra of the deinoxanthin were obtained by measuring the absorbance between 350nm and 600nm and were normalized by the dry cell weight (DCW) (Figure 17), the grey spectrum corresponding to a deinoxanthin standard (purified deinoxanthin extracted from *D. radiodurans*).

As shown in Figures 17 and 18, the overexpression of the endogenous farnesyl pyrophosphate synthase (FPPS) of *D. geothermalis* resulted in a dramatic increase of the deinoxanthin production by a factor two compared to the wild type strain (WT). This increased deinoxanthin production was correlated with a darker pink color of the *D. geothermalis* over-expressing the FPPS compared to the wild-type strain (Figure 18).

Example 13 : Production of cineol

A *Deinococcus geothermalis* strain was genetically engineered to produce cineole. A heterologous gene encoding a 1,8 cineole synthase (*CnsA*) from *Streptomyces clavuligerus* was inserted into chromosome replacing phosphotransacetylase (pta) gene. Expression of 1,8 cineole synthase gene was under the control of a constitutive promoter. The *D. geothermalis* K170G mutant of farnesyl pyrophosphate synthase (FPPS) gene was inserted into chromosome replacing the endogenous *fdps*. Overexpression of FDPS gene was under the control of a constitutive promoter. The resulting constructs were checked by sequencing. Seed cultures and cultures for cineole production were performed as detailed in example 1. After 24H or 48H of growth, 1 mL of culture was extracted by 0.5 mL of ethyl acetate. The organic phase was analyzed by GCMS. The production of cineole was identified and quantified on a Mass Spectrometer GC as detailed in example 1 for geraniol.

As shown in Figure 19, the insertion of 1,8 cineole synthase resulted in a cineole production by the recombinant *D. geothermalis*.

Example 14 : Production of limonene

A *Deinococcus geothermalis* strain was genetically engineered to produce
5 limonene. A heterologous gene encoding the limonene synthase (LS; GenBank:
AAD50304.1) gene from *Mentha longifolia*. The *M. longifolia* LS cDNA fused to GFP
was inserted into chromosome replacing phosphotransacetylase (pta) gene.
Overexpression of LS gene was under the control of a constitutive promoter. The *D.*
geothermalis K170G mutant of farnesyl pyrophosphate synthase (FPPS) gene was
10 inserted into chromosome replacing the endogenous *fdps*. Overexpression of FDPS gene
was under the control of a constitutive promoter. The resulting constructs were checked
by sequencing. Seed cultures and cultures for limonene production were performed as
detailed in example 1. After 24H or 48H of growth, 1 mL of culture was extracted by 0.5
mL of ethyl acetate. The organic phase was analyzed by GCMS. The production of
15 limonene was identified and quantified on a Mass Spectrometer GC as detailed in
example 1 for geraniol.

As shown in Figure 20, the insertion of limonene synthase resulted in a limonene production by the recombinant *D. geothermalis*.

Example 15 : Production of linalool

20 A *Deinococcus geothermalis* strain was genetically engineered to produce
linalool. A heterologous gene encoding the linalool synthase (LIS) gene from *Perilla*
frutescens. The LIS cDNA fused to GFP was inserted into chromosome replacing
phosphotransacetylase (pta) gene. Overexpression of LIS gene was under the control of a
constitutive promoter. The *D. geothermalis* K170G mutant of farnesyl pyrophosphate
25 synthase (FPPS) gene was inserted into chromosome replacing the endogenous *fdps*.
Overexpression of FDPS gene was under the control of a constitutive promoter. The
resulting constructs were checked by sequencing. Seed cultures and cultures for linalool
production were performed as detailed in example 1. The production of linalool was
identified and quantified on a Mass Spectrometer GC as detailed in example 1 for
30 geraniol.

As shown in Figure 21, the insertion of linalool synthase resulted in a linalool production by the recombinant *D. geothermalis*.

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CLAIMS

1. A method of producing a terpene or terpenoid comprising (i) culturing a recombinant *Deinococcus* bacterium that is genetically modified to increase 1-deoxyxylulose 5-phosphate synthase (DXS) activity and/or isopentenyl pyrophosphatase
5 isomerase (IPP isomerase) activity, under conditions suitable to produce the terpene or terpenoid and optionally (ii) recovering the terpene or terpenoid.
2. The method of claim 1, wherein the recombinant *Deinococcus* bacterium overexpresses a native, homologous or heterologous *idi* gene.
3. The method of claim 1 or 2, wherein the recombinant *Deinococcus* bacterium
10 overexpresses a native, homologous or heterologous *dxs* gene.
4. The method of any of claims 1 to 3, wherein the recombinant *Deinococcus* bacterium expresses an improved DXS enzyme.
5. The method of claim 4, wherein the improved DXS enzyme is a mutant DXS comprising a cysteine at position corresponding to position 244 of SEQ ID NO: 52.
- 15 6. The method of claim 4, wherein the recombinant *Deinococcus* bacterium expresses a gene encoding R244C mutant of the DXP synthase from *D. radiopugnans* (SEQ ID NO: 8), a gene encoding R238C mutant of the DXP synthase from *D. yunweiensis* (SEQ ID NO: 14) or a gene encoding R241C mutant of the DXP synthase from *D. geothermalis* (SEQ ID NO: 56).
- 20 7. The method of any of claims 1 to 6, wherein the recombinant *Deinococcus* bacterium expresses an improved DXS enzyme and overexpresses a native, homologous or heterologous *idi* gene.
8. The method of any of claims 1 to 7, wherein the recombinant *Deinococcus* bacterium overexpresses a native, homologous or heterologous gene encoding FPP
25 synthase.
9. The method of claim 8, wherein the gene encoding FPP synthase encodes a polypeptide selected from the group consisting of

- a) a polypeptide comprising all or an active part of the amino acid sequence of SEQ ID NO: 47;
- b) a polypeptide having an amino acid sequence having at least 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% sequence identity to SEQ ID NO: 47;
- 5 c) a polypeptide encoded by a nucleotide sequence having at least 60, 65, 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% sequence identity to SEQ ID NO: 46; or
- d) a polypeptide which is encoded by a nucleic acid sequence which is capable of hybridizing under medium/high stringency, preferably high or very high, conditions with (i) the nucleic acid sequence set forth in SEQ ID NO: 46, (ii) its complementary strand,
10 or (iii) a subsequence of (i) or (ii).
10. The method of claim 8 or 9, wherein the FPP synthase further exhibits dimethylallyltransferase activity and/or geranylgeranyl diphosphate synthase activity.
11. The method of any of claims 1 to 10, wherein, in the recombinant *Deinococcus* bacterium, at least one gene selected from the group consisting of native, homologous or
15 heterologous *dxr*, *ispD*, *ispE*, *ispF*, *ispG* and *ispH* genes, is overexpressed.
12. The method of any of claims 1 to 11, wherein the recombinant *Deinococcus* bacterium further comprises a gene encoding a heterologous terpene synthase.
13. The method of claim 12, wherein the heterologous terpene synthase is selected from the group consisting of monoterpene synthases, diterpene synthases, triterpene
20 synthases and sesquiterpene synthases.
14. The method of claim 12 or 13, wherein the heterologous terpene synthase is a monoterpene synthase, preferably a geraniol synthase, a cineole synthase, a limonene synthase or a linalool synthase.
15. The method of claim 12 or 13, wherein the heterologous terpene synthase is a
25 sesquiterpene synthase, preferably a (+)-epi-alpha-bisabolol synthase.
16. The method of any one of claims 1 to 15, wherein, in the recombinant *Deinococcus* bacterium, the gene encoding the lycopene beta-cyclase is inactivated.

17. The method of any one of claims 1 to 16, wherein the terpene or terpenoid is selected from monoterpenes, diterpenes, triterpenes, sesquiterpenes and carotenoids.

18. The method of any one of claims 1 to 17, wherein the terpene or terpenoid is a sesquiterpene.

5 19. The method of any one of claims 1 to 17, wherein the terpene or terpenoid is a carotenoid.

20. The method of claim 19, wherein the carotenoid is lycopene or any other carotenoid compound derived from lycopene.

21. The method of claim 19 or 20, wherein the carotenoid is deinoxanthine.

10 22. A recombinant *Deinococcus* bacterium as defined in any one of claims 1 to 16.

Figure 1

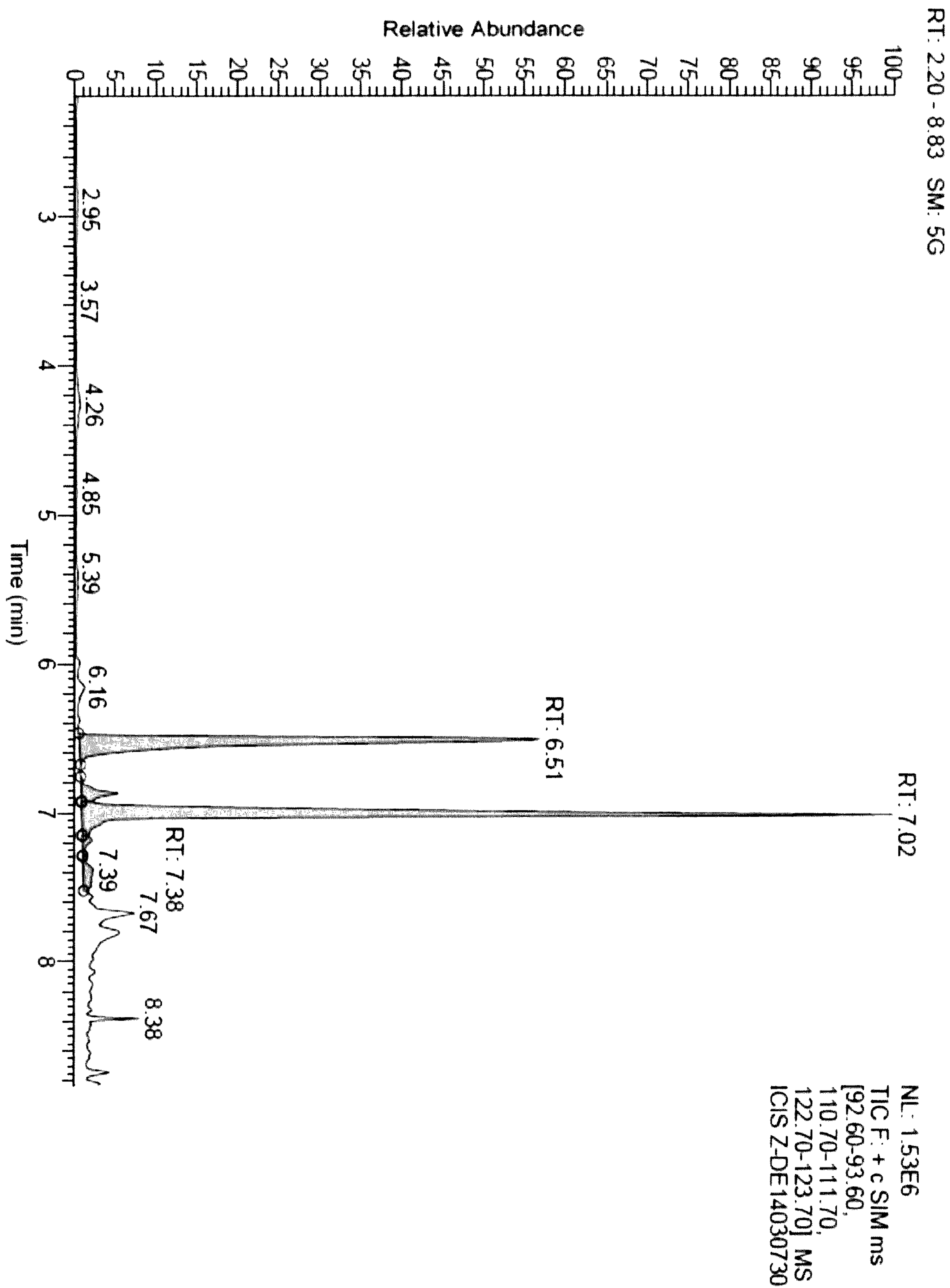


Figure 2

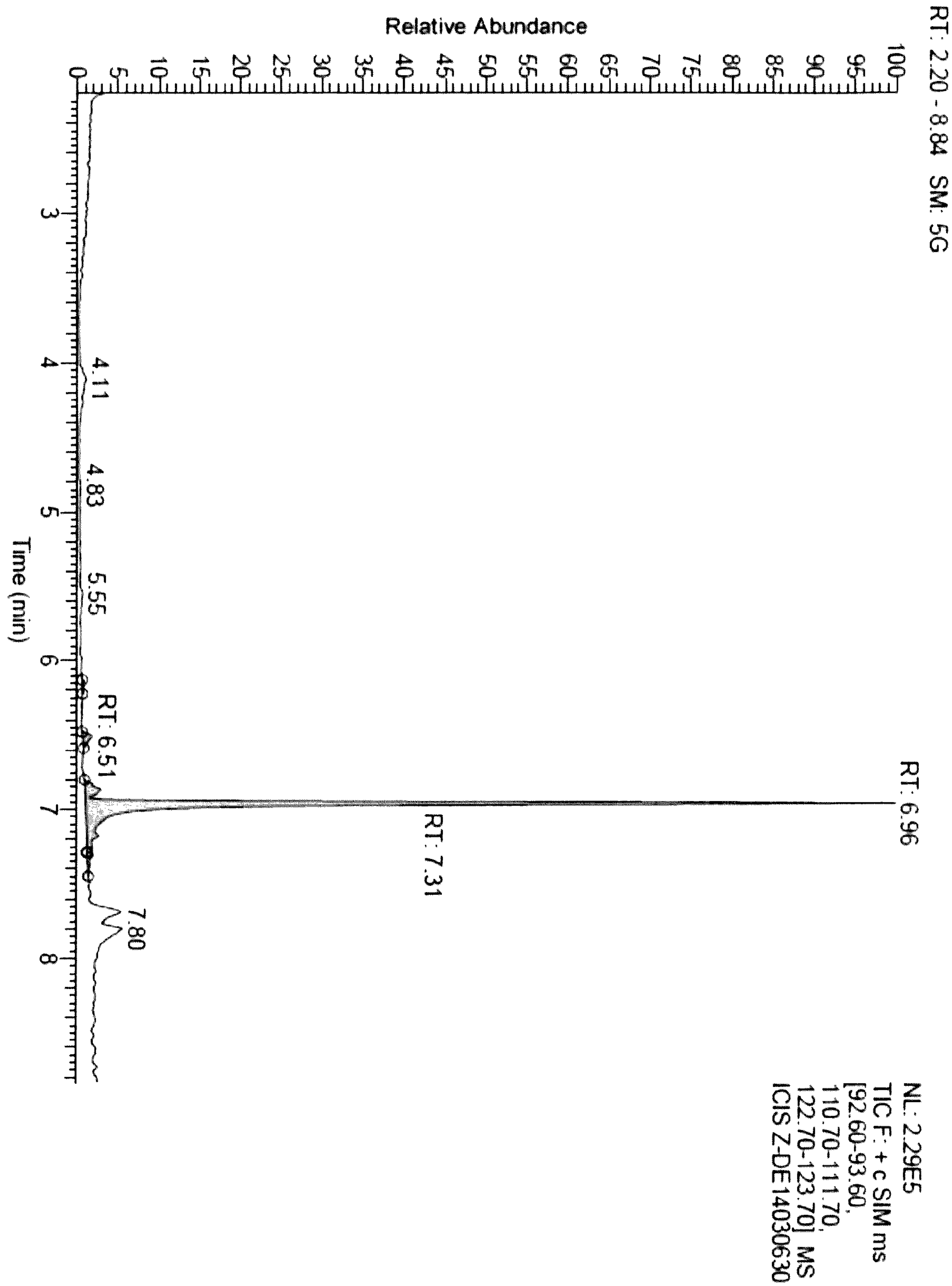


Figure 3

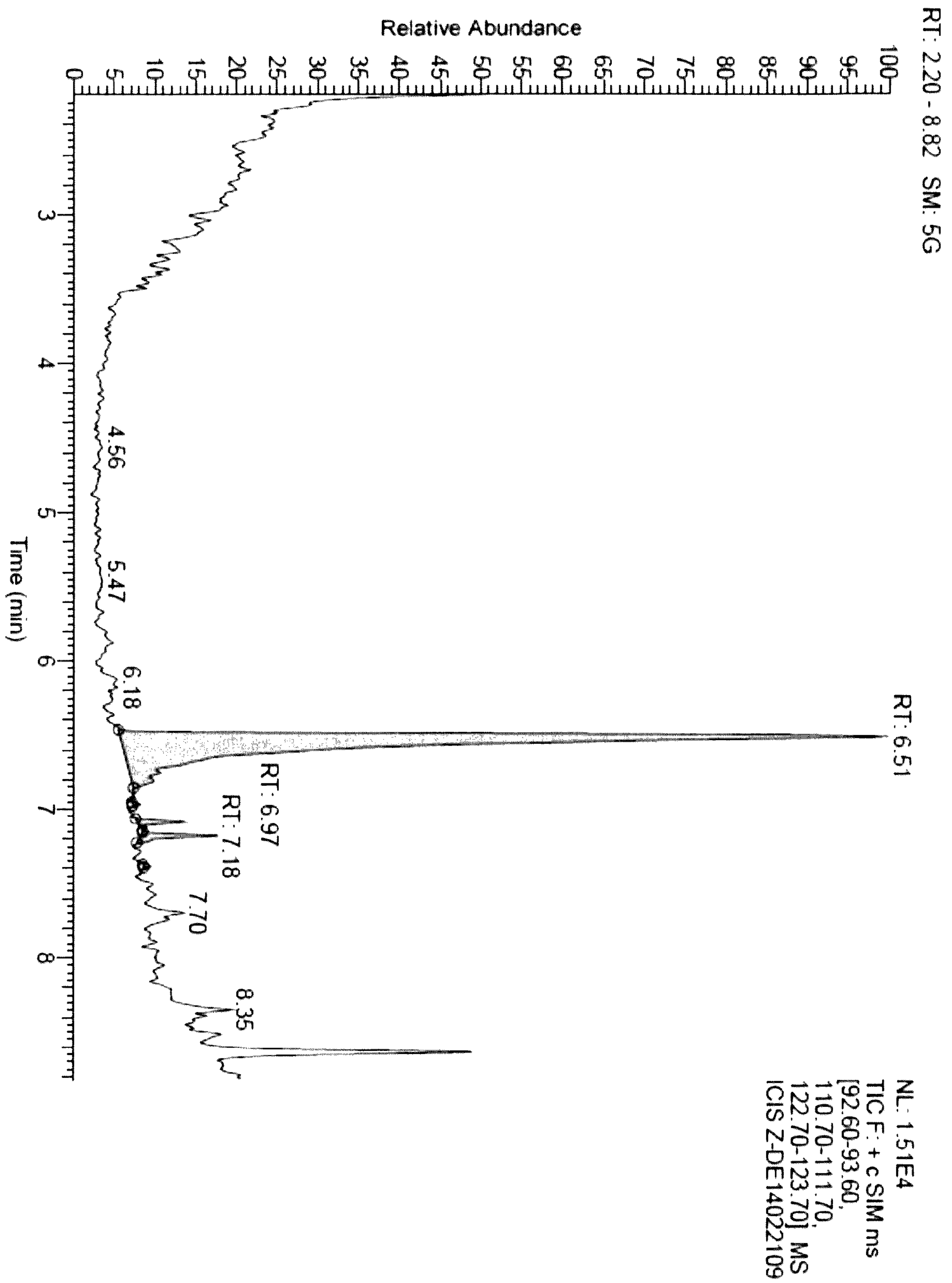


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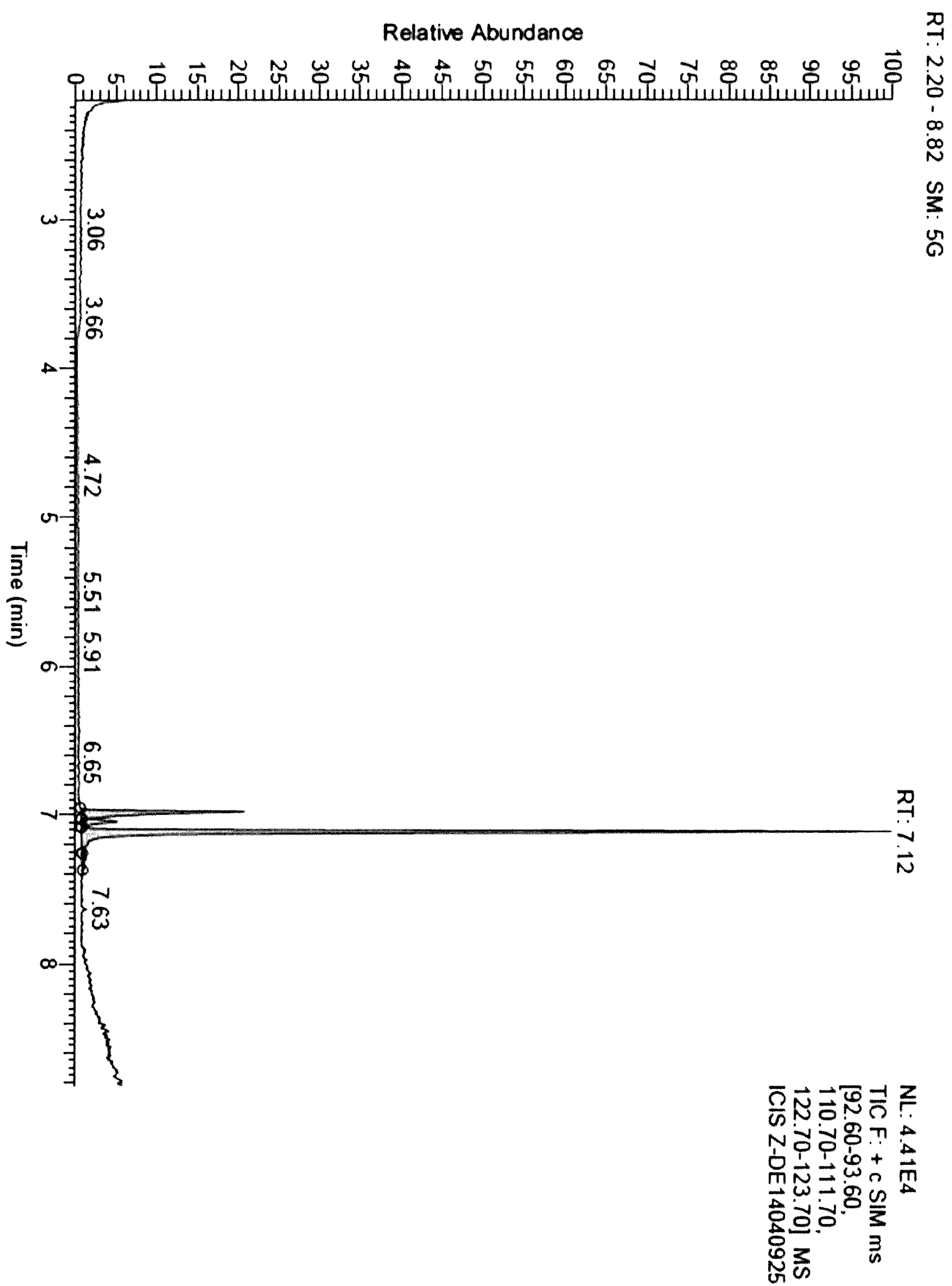
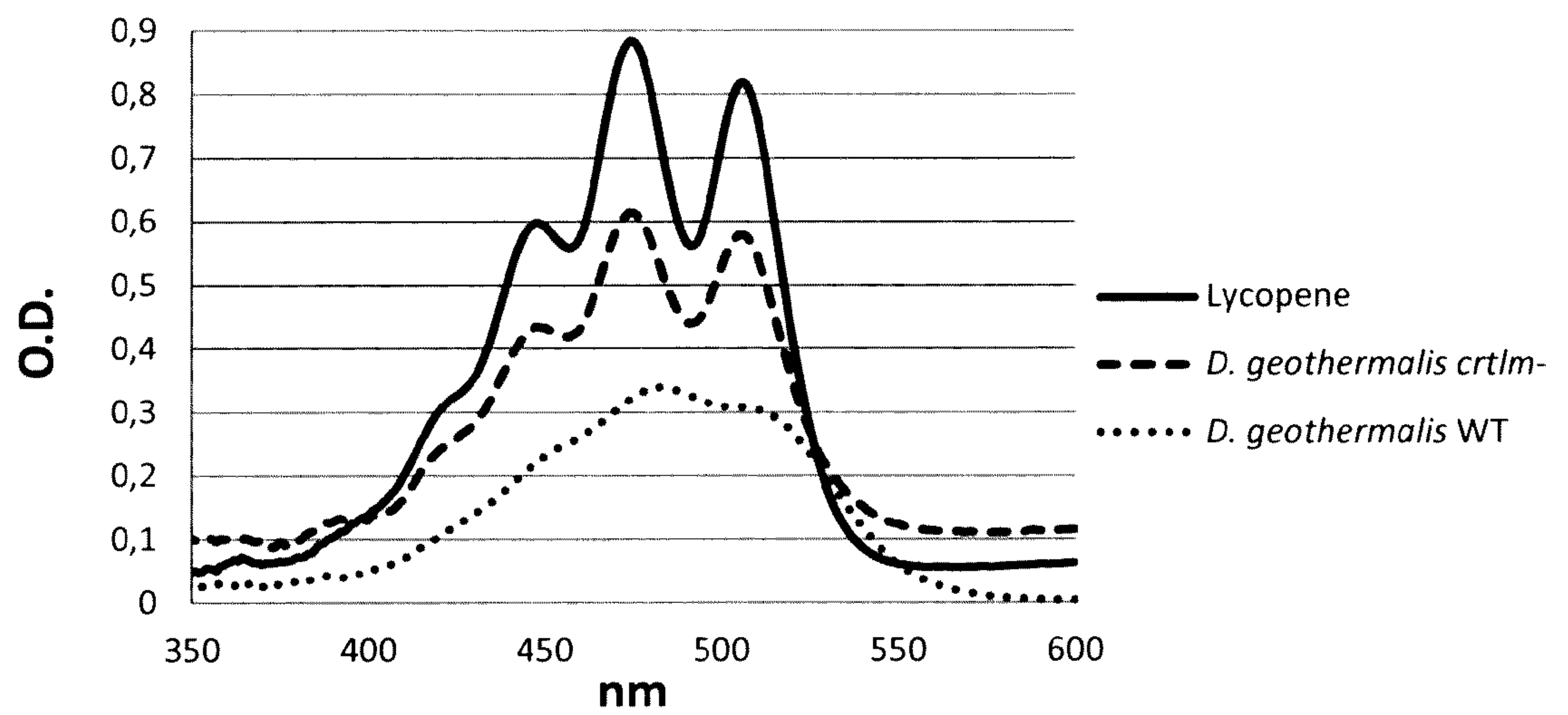
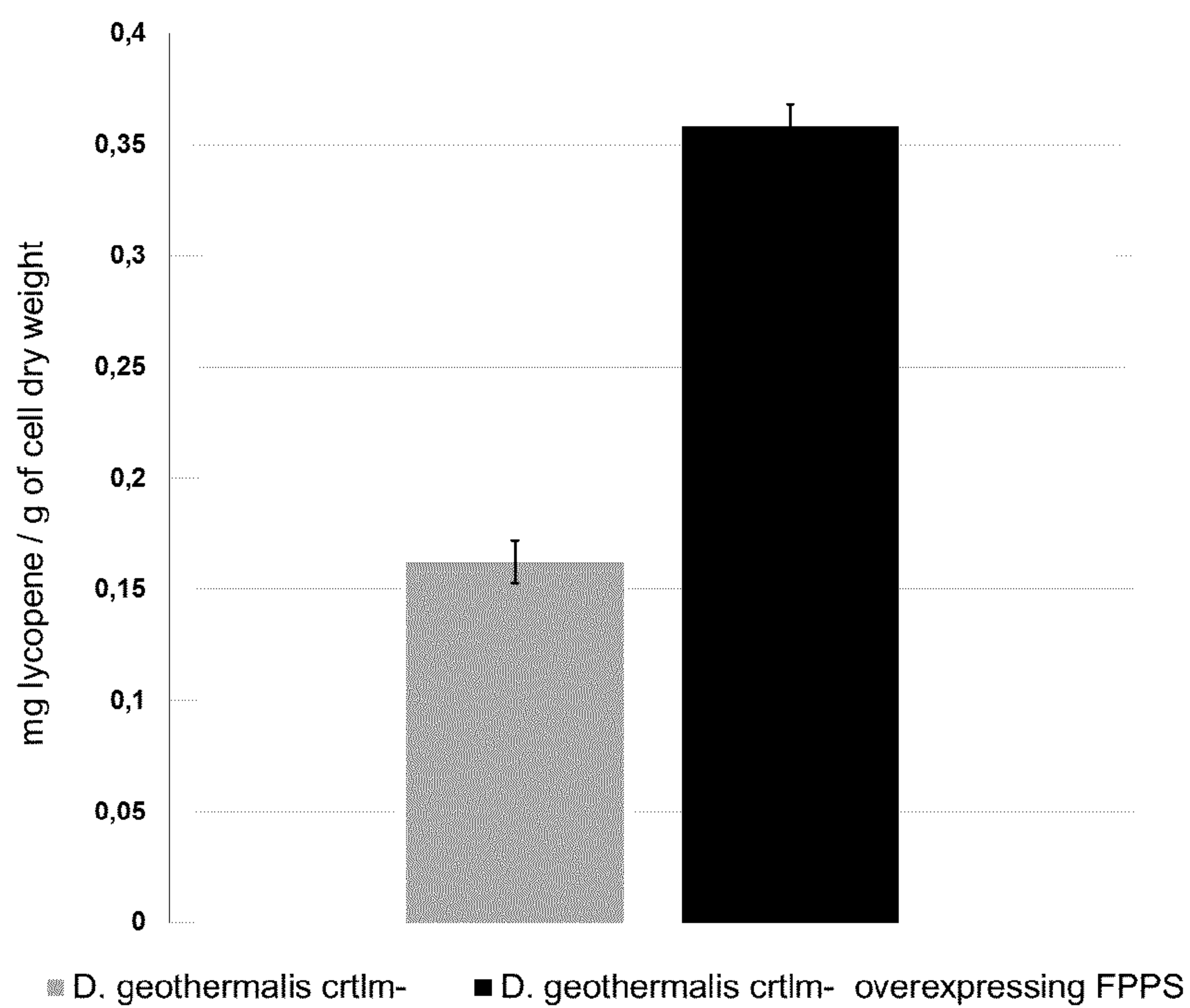
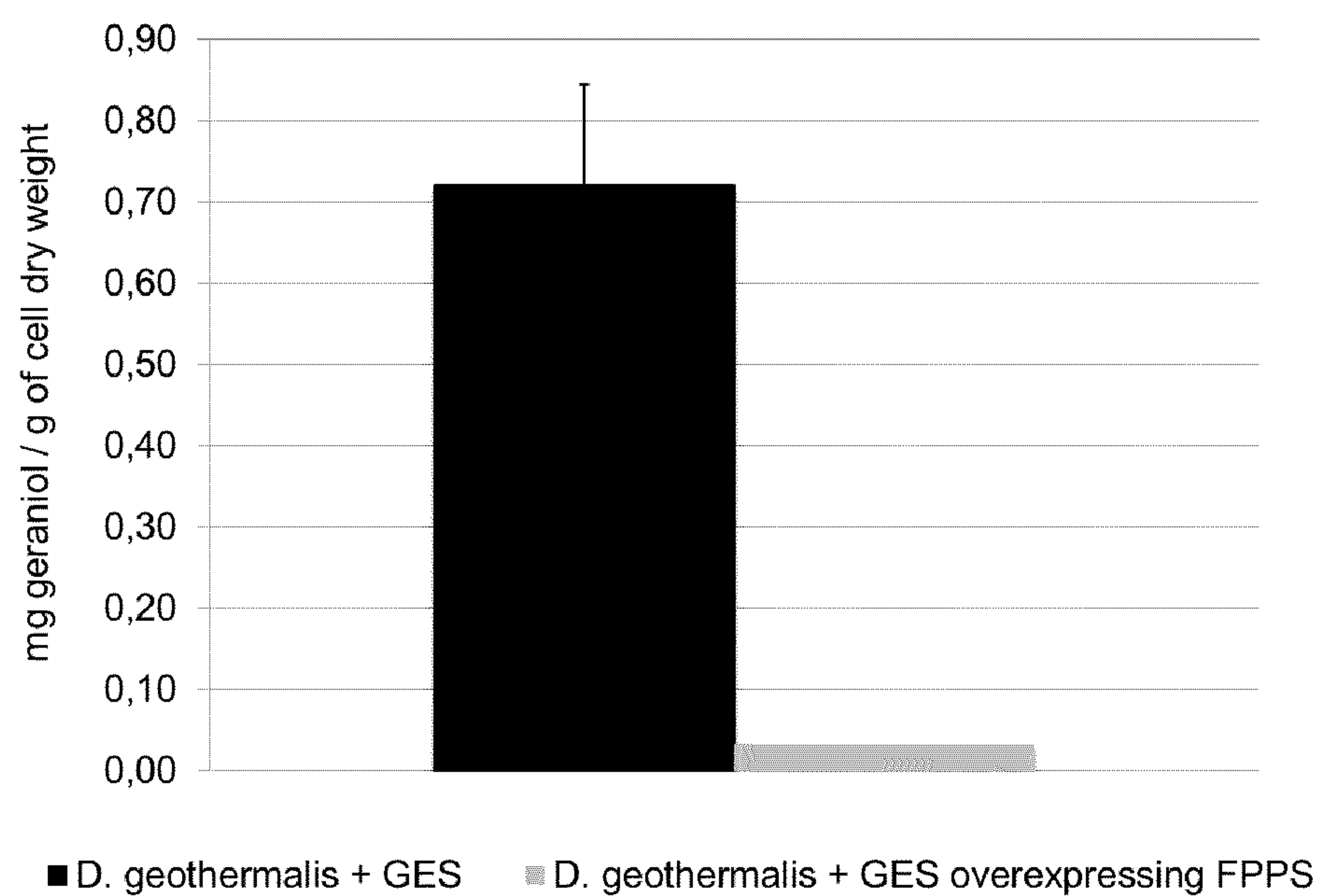


Figure 5

**Figure 6**

**Figure 7****Figure 8**

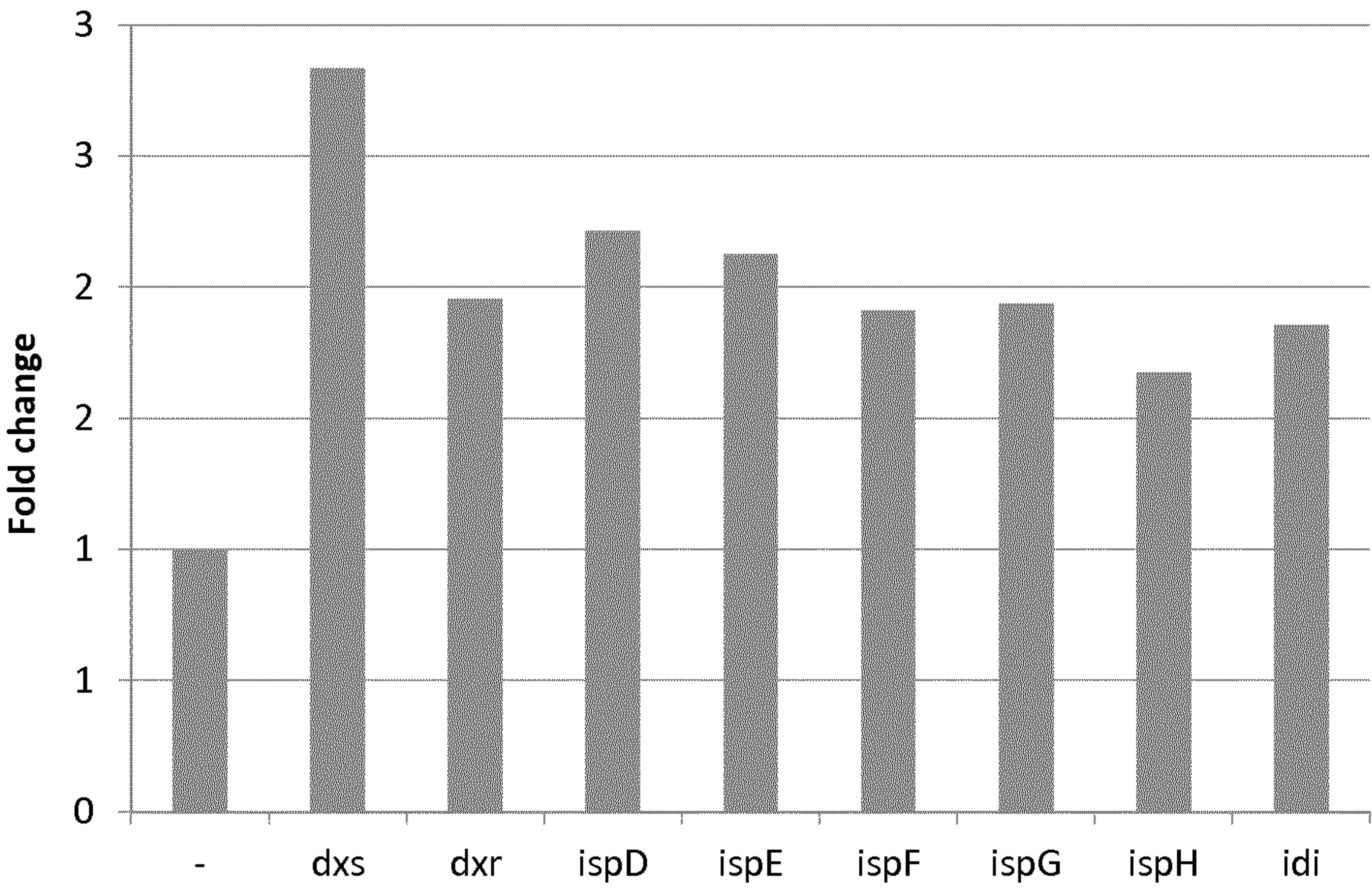


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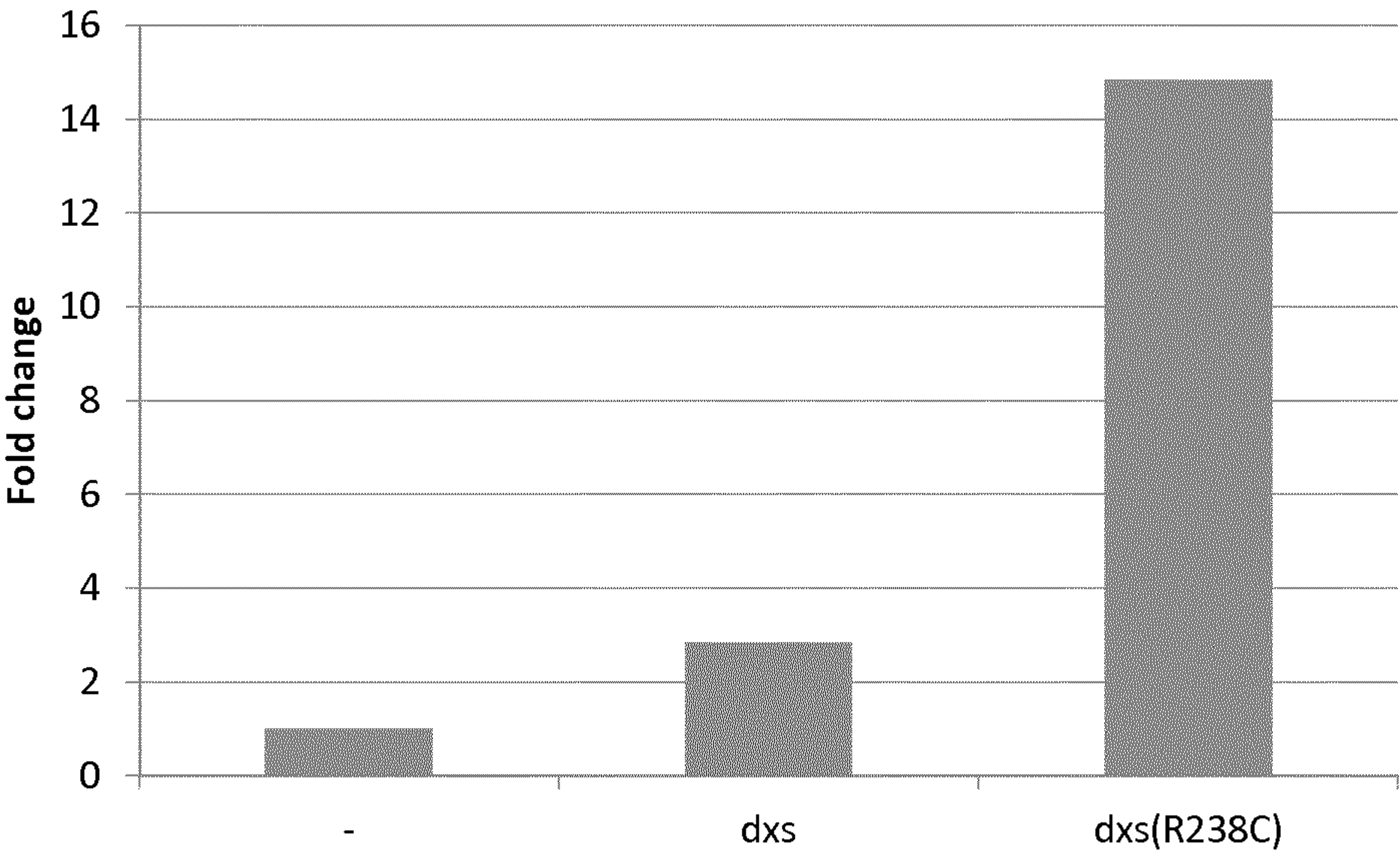
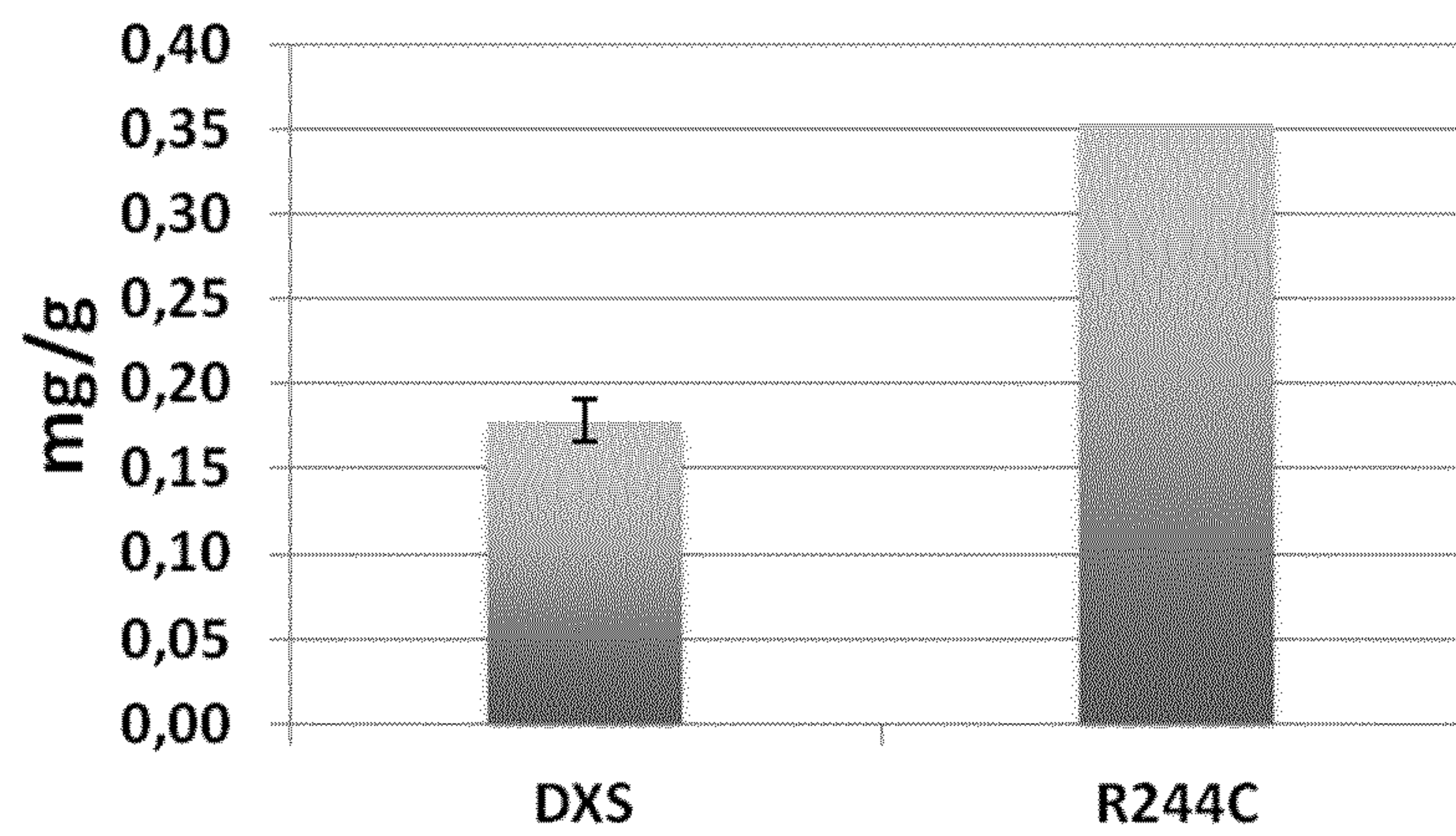
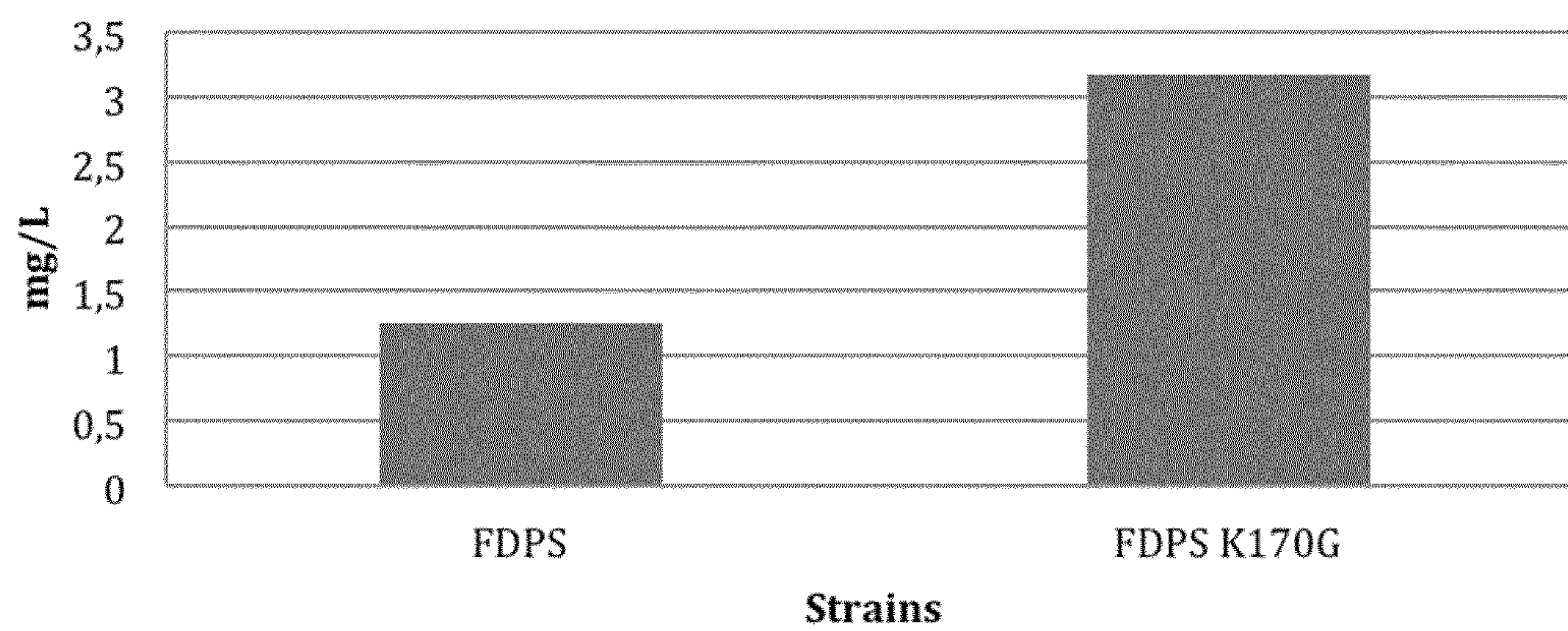


Figure 10

**Figure 11****Figure 12**

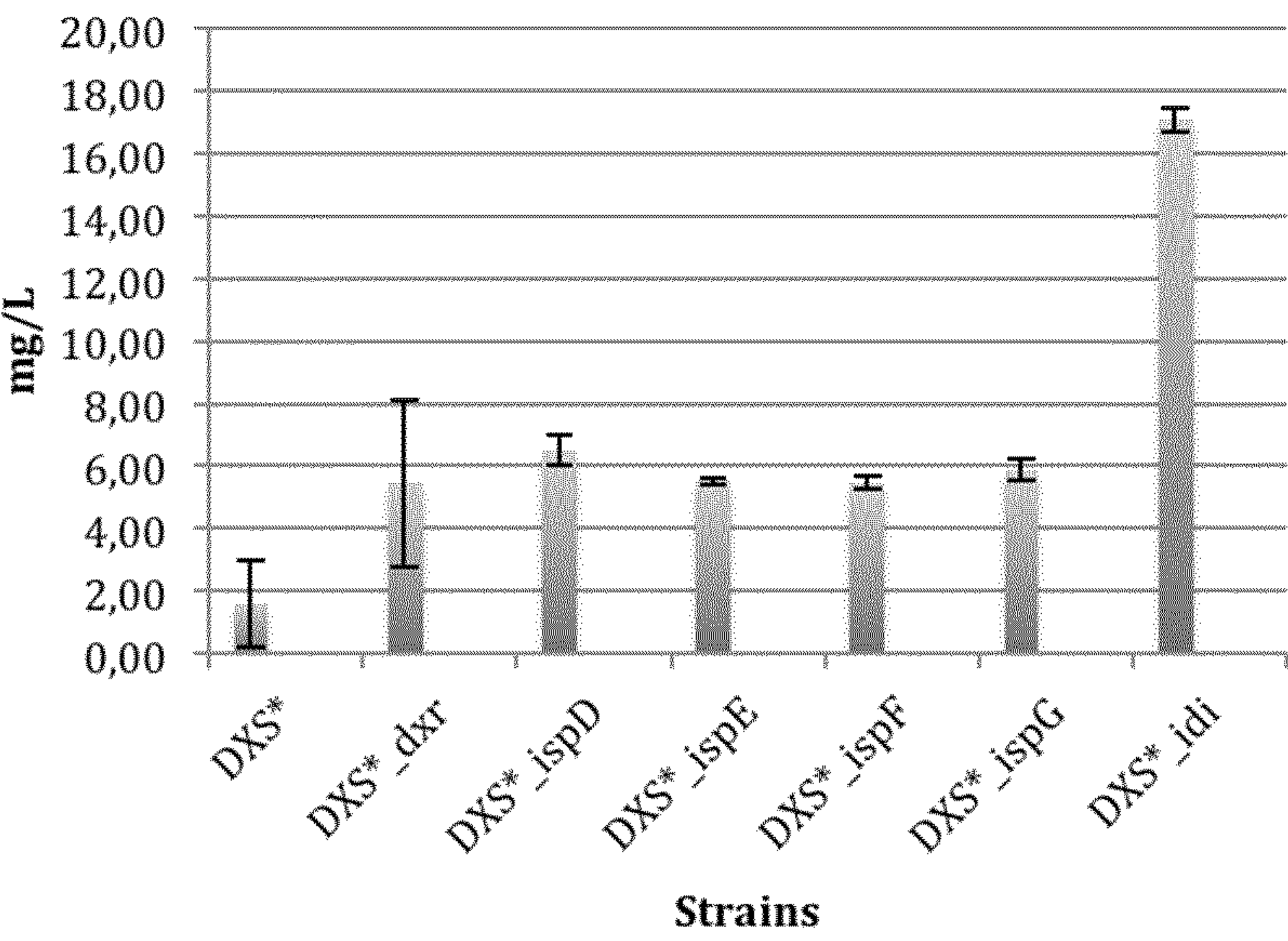


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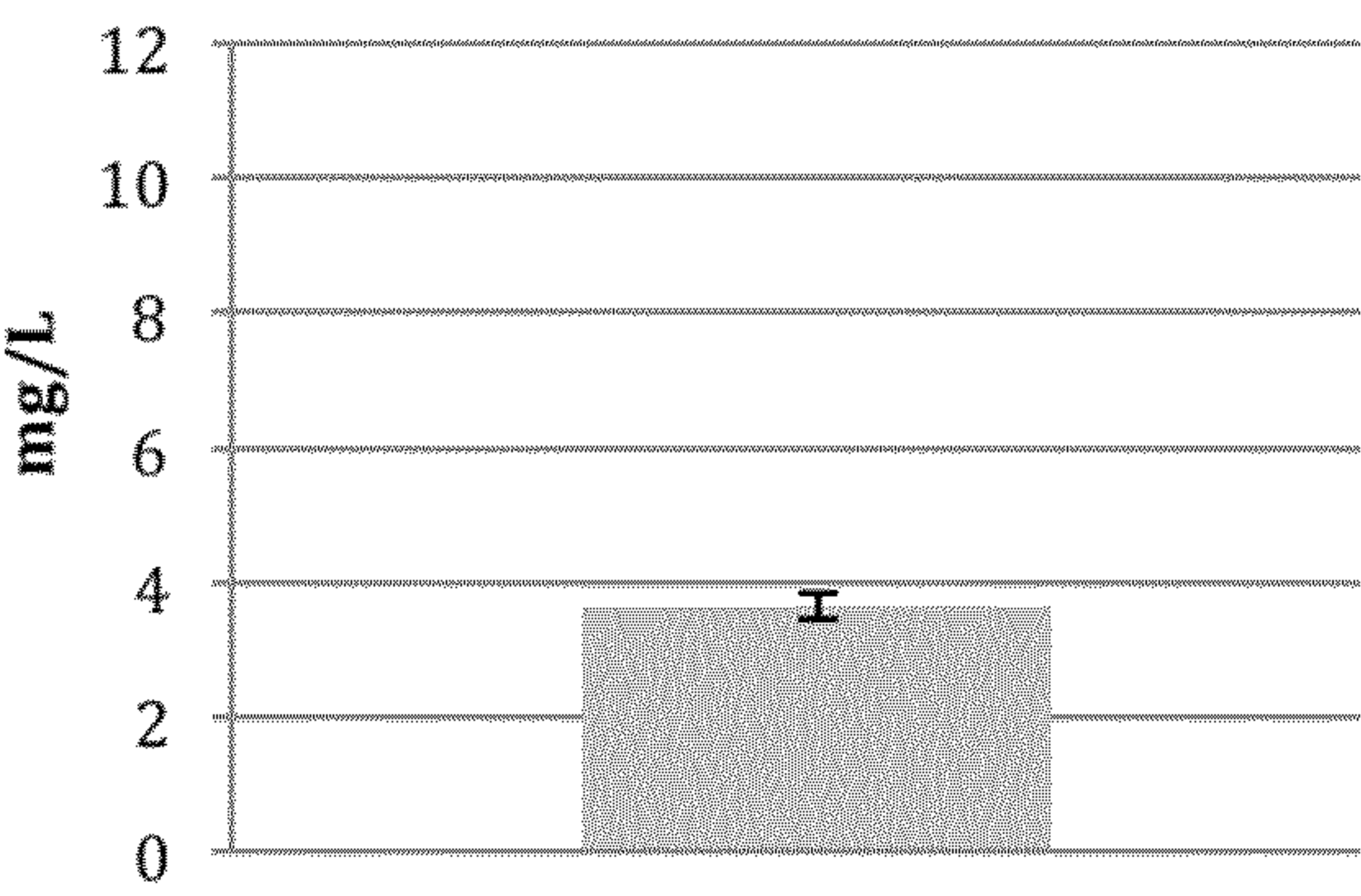
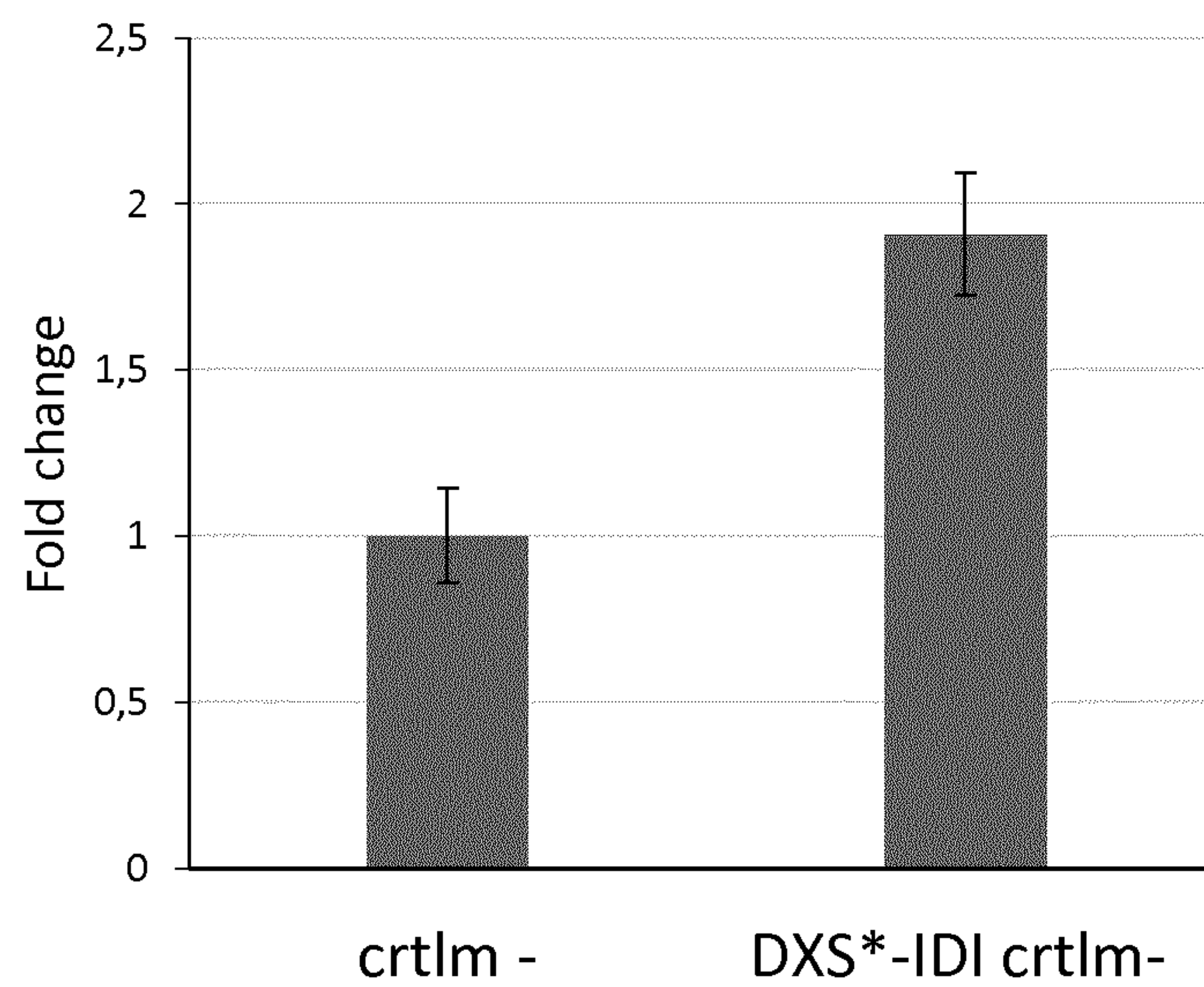
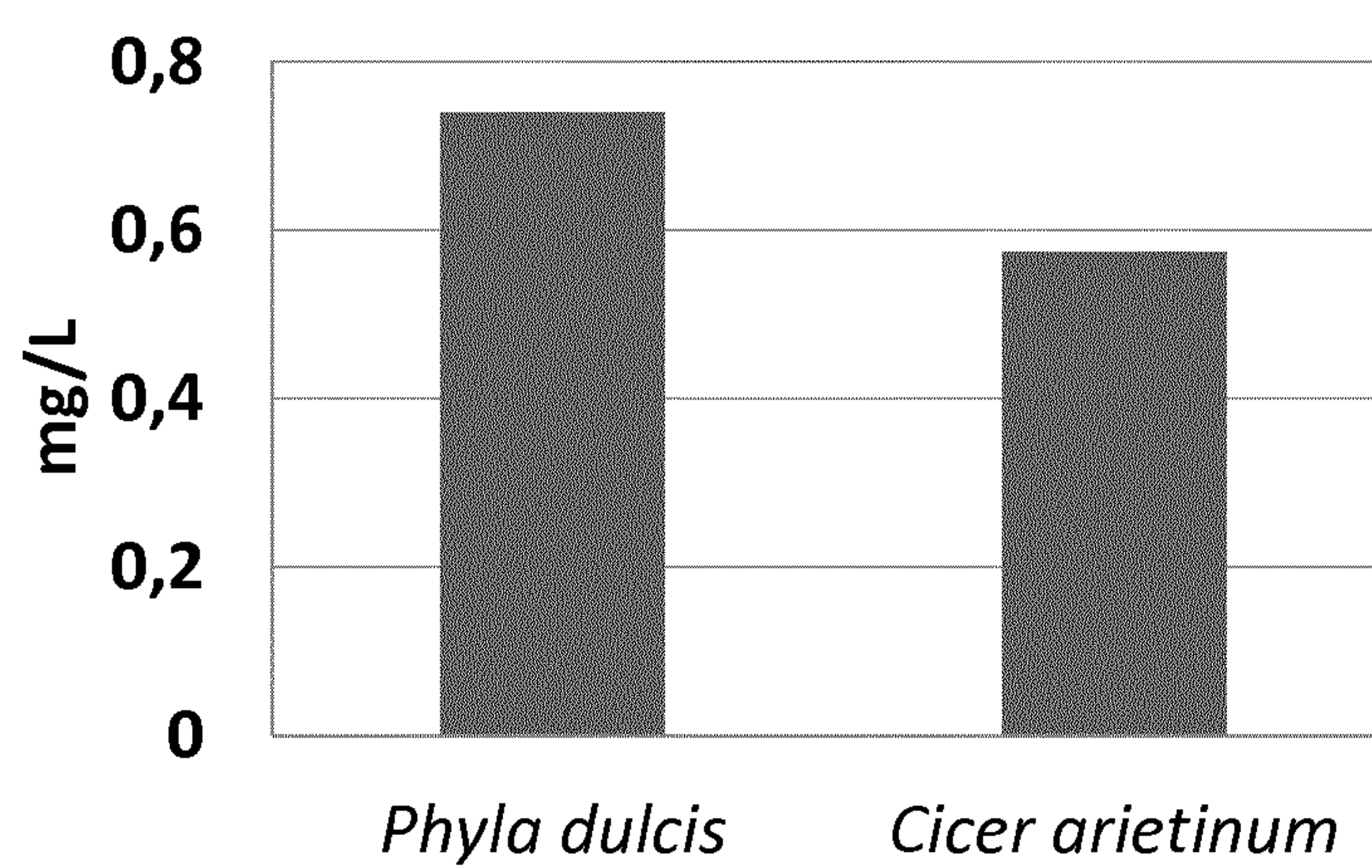
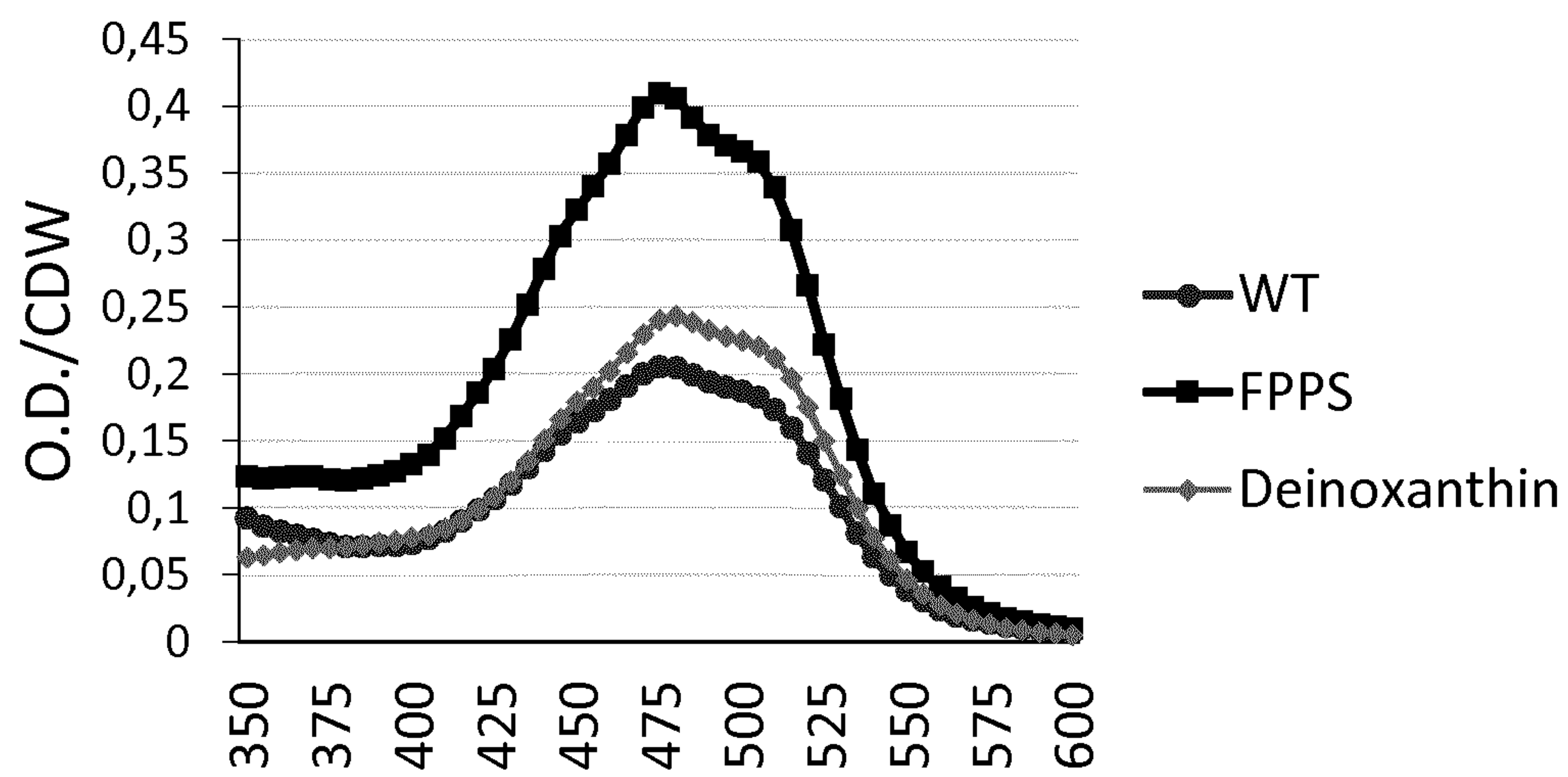
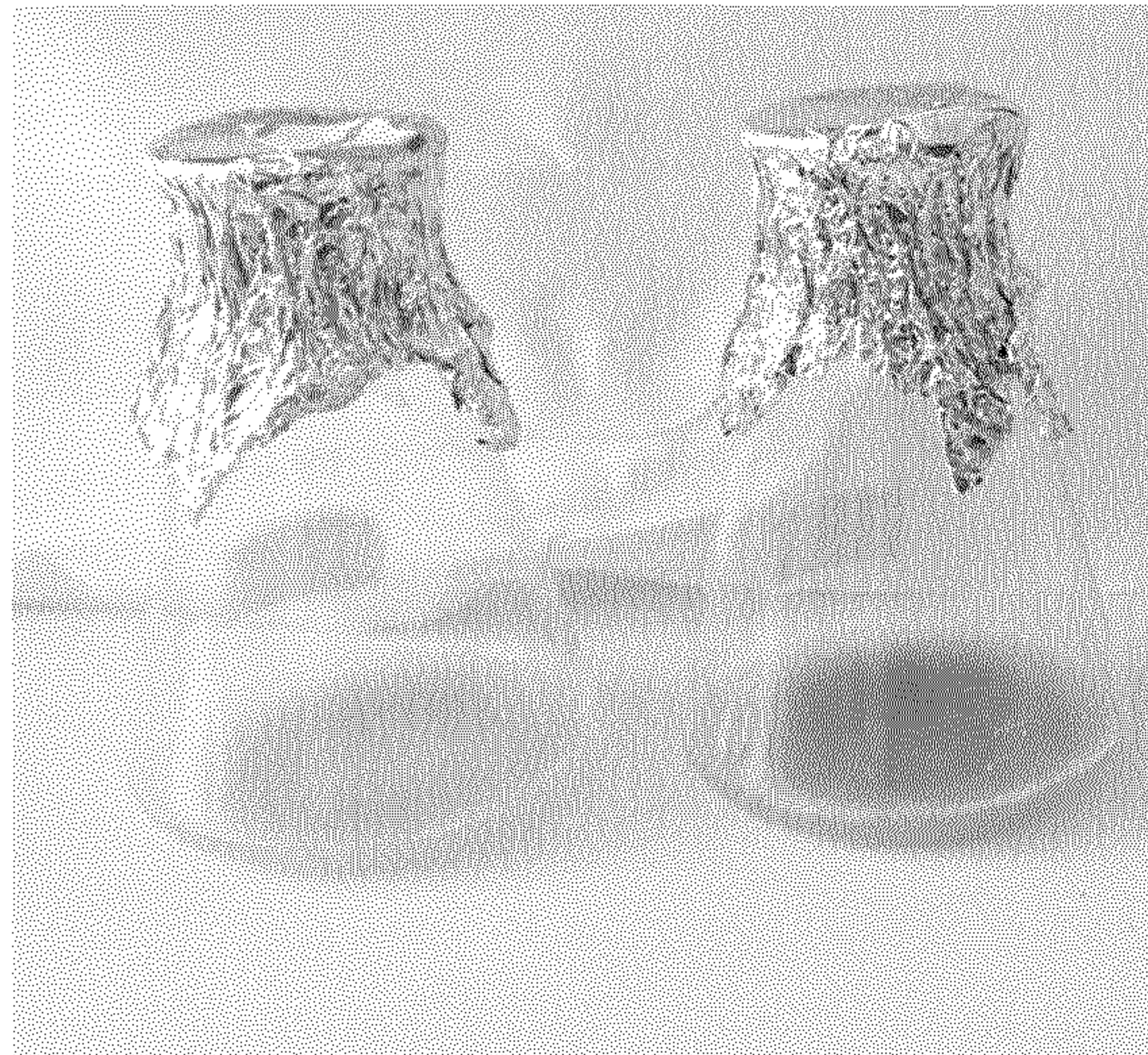


Figure 14

**Figure 15****Figure 16**

**Figure 17****Figure 18**

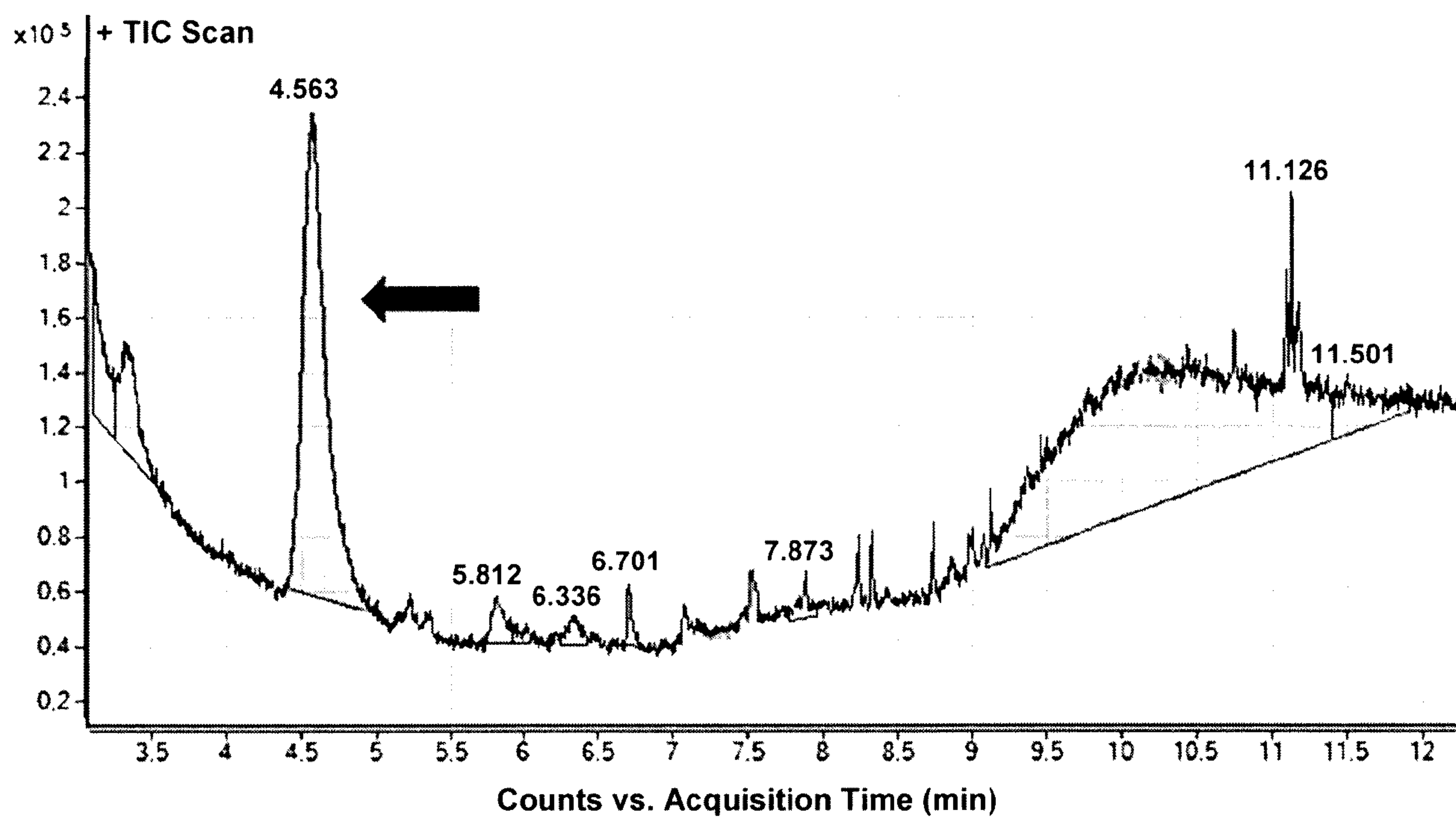


Figure 19

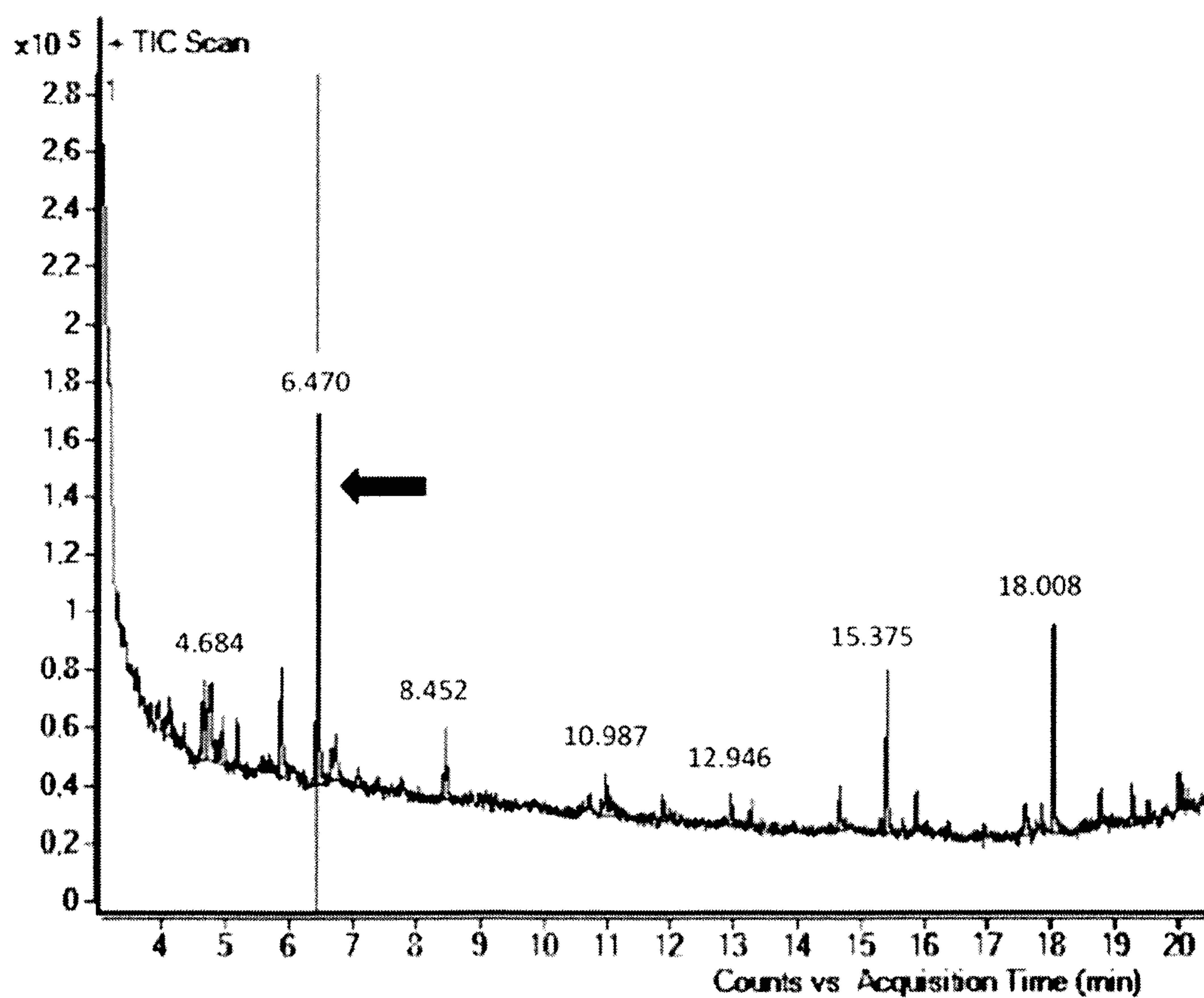
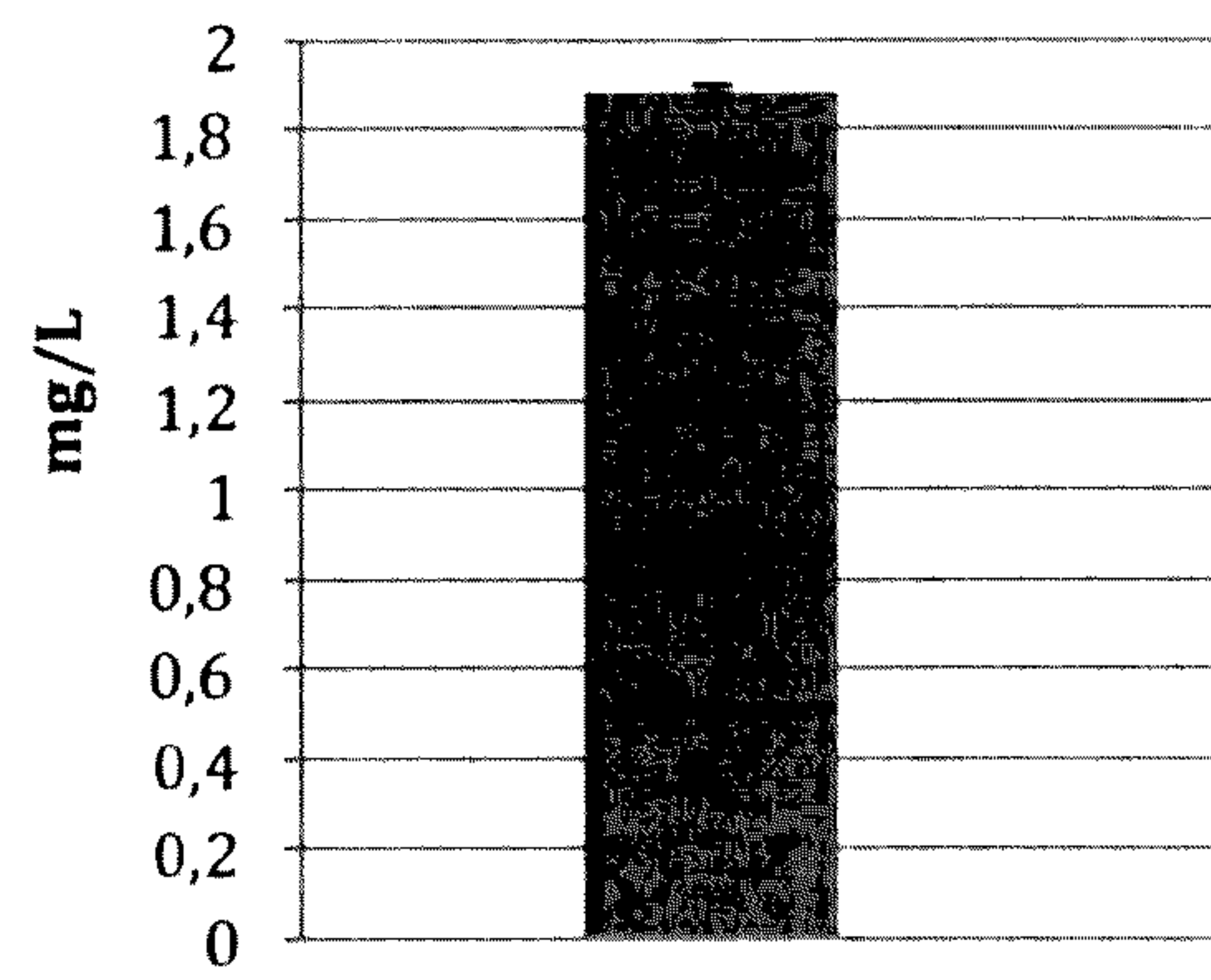


Figure 20

**Figure 21**