The present invention relates to an isolated microorganism which is Cupriavidus basilensis strain HMF14 Deposit number DSM 22875, and its use in a process for the in-situ detoxification of lignocelluloses hydrolysate.
— with (an) indication(s) in relation to deposited biological material furnished under Rule 13bis separately from the description (Rules 13bis.4(d)(i) and 48.2(a)(viii))
— with sequence listing part of description (Rule 5.2(a))
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N1/20 C12N15/31 C12N15/52 C12N9/00 C12N9/02
C12N9/16 C12N9/88 C07K14/195 A62D3/02
ADD. C12R1/01

According to International Patent Classification (IPC) and/or both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12R C12N C12P C07K A62D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of database and, where practical, search terms used)
EPO-Internal, Sequence Search, BIOSIS, EMBASE, WPI Data, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>A</td>
<td>the whole document</td>
<td>27-35, 39, 44, 45</td>
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</table>

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle of the theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "S" document: member of the same patent family

Date of the actual completion of the international search: 8 June 2011
Date of mailing of the international search report: 24/06/2011

Name and mailing address of the ISA:
European Patent Office, P.B. 5818 Patentilaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040
Fax: (+31-70) 340-3016

Authorized officer:
Dumont, Eilisabeth

Form PCT/ISA/210 (second sheet) (April 2005)
DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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</table>
| A        | OKUDA N ET AL: "Biological detoxification of waste house wood hydrolysate using
             urea biomass thermosphaeria culture in 25-36,
             ethanol production", JOURNAL OF BIOSCIENCE AND BIOENGINEERING
             AUGUST 2008 ELSEVIER NL,
             vol. 106, no. 2, August 2008 (2008-08), pages 128-133,
             abstract |
| A        | HOU-RUI ZHANG ET AL: "Novel isolates for biological detoxification of
             lignocellulosic hydrolysate", APPLIED BIOCHEMISTRY AND
             BIOTECHNOLOGY, 38-46 vol. 152, no. 2, February
             2009 (2009-02), pages 199-212, ISSN: 0273-2289, the
             whole document |
| A        | KÖENIG K ET AL: "Xanthine dehydrogenase and 2-furoyl-CoA dehydrogenase from
             Pseudomonas putida Ful: two novel structural compositions,
             "", JOURNAL OF BACTERIOLOGY OCT 1990,
             vol. 172, no. 10, October 1990 (1990-10), pages
             5999-6009, ISSN: 0021-9193, abstract |
| A        | DATABASE UniProt: 22 August 2008 (2008-08-22), Nicholos N.N. et al.: "SubName: Ful=PsF1", XP002569760, Database access on no. B3FN0, the whole document |

abstract
figure 1
page 54, right-hand column, paragraph 2

/-/-
<table>
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<th>Category</th>
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| X        | DATABASE EMBL [Online] 23 June 2008 (2008-06-23), "CH0_0F4424xnl8rl.abl CH0_0F4 N. coti ana
tabacum genomic DNA", genomic survey sequence. ",
XP002640258, retrieved from EBI access no. EM_GSS: FH310161
Database access no. FH310161 the whole document
invention on 4; 68% identity with SEQ ID NO: 20 | 10,27-36 |
| X        | DATABASE UniProt [Online] 29 April 2008 (2008-04-29), "SubName:
Ful 1=3-octaprenyl-4-hydroxybenzoate
hydroxy-lyase; ",
XP002640259, retrieved from EBI access no. UNI_PR0T: B1FTX3
Database access no. B1FTX3 the whole document
invention on 5; 78% identity with SEQ ID NO: 21 | 11, 12, 27-36 |
Global-Ocean-Sampling_GS-33-01-01-IP3-IP8K
B marine metagenome genomic clone
1061006227953 5', genomic survey sequence. ",
XP002640260, retrieved from EBI access no. EM_GSS: ER268392
Database access no. ER268392 the whole document
invention on 5; 65.2% identity with SEQ ID NO: 22 | 39-45 |
<p>| X        | DATABASE EMBL [Online] | 12,27-36 |</p>
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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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INTERNATIONAL SEARCH REPORT

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
   a. (means)
      - [ ] on paper
      - [x] in electronic form
   b. (time)
      - [x] in the international application as filed
      - [ ] together with the international application in electronic form
      - [ ] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Form PCT/ISA/21 0 (continuation of first sheet (1)) (July 2009)
**INTERNATIONAL SEARCH REPORT**

**Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. □ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. □ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

   1-4, 9-14, 25, 26, 38 (completely); 27-36, 39-46 (partially)

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

□ The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.

□ The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.
This International Searching Authority found multiple (groups of) inventions in this International application, as follows:

1. claims: 1-4, 38(completely); 36, 40-43, 46(parially)
   
   Cupriavidus basi lens strain HMF14 deposited as DSM 22875, polypeptides and polynucleotides of said strain and subject-matter related to the strain and/or the polypeptides and polynucleotides.

2. claims: 5, 6(completely); 27-36, 39-42 (partially)
   
   Polypeptide having aldehyde dehydrogenase activity, which comprises the amino acid sequence of SEQ ID No. 15, encoded by the nucleotide sequence of SEQ ID No. 16; subject-matter related thereto.

3. claims: 7, 8(completely); 27-36, 39-42 (partially)
   
   Polypeptide having LysR family transcriptional regulator activity, which comprises the amino acid sequence of SEQ ID No. 17, encoded by the nucleotide sequence of SEQ ID No. 18; subject-matter related thereto.

4. claims: 9, 10(completely); 27-36, 39-45 (partially)
   
   Polypeptide having 2,5-furan dicarboxylic acid decarboxylase activity, which comprises the amino acid sequence of SEQ ID No. 19, encoded by the nucleotide sequence of SEQ ID No. 20; subject-matter related thereto.

5. claims: 11, 12(completely); 27-36, 39-45 (partially)
   
   Polypeptide having 2,5-furan dicarboxylic acid decarboxylase activity, which comprises the amino acid sequence of SEQ ID No. 21, encoded by the nucleotide sequence of SEQ ID No. 22; subject-matter related thereto.

6. claims: 13, 14(completely); 27-36, 39-43 (partially)
   
   Polypeptide having HMF/furfural oxidoreductase activity, which comprises the amino acid sequence of SEQ ID No. 23, encoded by the nucleotide sequence of SEQ ID No. 24; subject-matter related thereto.

7. claims: 15, 16(completely); 27-36, 39-42 (partially)
   
   Polypeptide having LysR type transcriptional regulator activity, which comprises the amino acid sequence of SEQ ID No. 25, encoded by the nucleotide sequence of SEQ ID No. 26; subject-matter related thereto.
No. 33, encoded by the nucleotide sequence of SEQ ID No. 34; subject-matter related thereto.

---

8. claims: 17, 18 (completely) ; 27-36, 39-45 (partially)

Polypeptide having furoyl-CoA dehydrogenase (large subunit) activity, which comprises the amino acid sequence of SEQ ID No. 35, encoded by the nucleotide sequence of SEQ ID No. 36; subject-matter related thereto.

---

9. claims: 19, 20 (completely) ; 27-36, 39-45 (partially)

Polypeptide having furoyl-CoA dehydrogenase (FAD binding subunit) activity, which comprises the amino acid sequence of SEQ ID No. 37, encoded by the nucleotide sequence of SEQ ID No. 38; subject-matter related thereto.

---

10. claims: 21, 22 (completely) ; 27-36, 39-45 (partially)

Polypeptide having furoyl-CoA dehydrogenase 2Fe-2S iron sulfur subunit activity, which comprises the amino acid sequence of SEQ ID No. 39, encoded by the nucleotide sequence of SEQ ID No. 40; subject-matter related thereto.

---

11. claims: 23, 24 (completely) ; 27-36, 39-44 (partially)

Polypeptide having furoyl-CoA synthase activity, which comprises the amino acid sequence of SEQ ID No. 41, encoded by the nucleotide sequence of SEQ ID No. 42; subject-matter related thereto.

---

12. claims: 25, 26 (completely) ; 27-36, 39-44 (partially)

Polypeptide having 2-oxoglutarate dehydrogenase activity, which comprises the amino acid sequence of SEQ ID No. 43, encoded by the nucleotide sequence of SEQ ID No. 44; subject-matter related thereto.

---

13. claims: 37 (completely) ; 40-42 (partially)

Process for the in situ detoxification of lignocellulose hydrolysate containing furanic compounds by contacting the lignocellulose hydrolysate with a suitable host microorganism containing enzymes from a microorganism from the family Burkholderiaceae, which convert furanic compounds.

---

14. claim: 44 (partially)
Process for the conversion of HMF, HMF alcohol, HMF acid and/or 2,5-furandicarboxylic acid to 2-furoyl-CoA comprising furfuryl alcohol or furfural with furoyl-CoA-dehydrogenase, furoyl-CoA-synthase, 2-oxoglutaroyl-CoA-hydrolase and 2,5-furandicarboxylic acid decarboxylase catalyst in the presence of a coenzyme cofactor.

---

15. Claim: 46(partially)

Method for the biocatalytical production of biofuels such as ethanol in a host microorganism having the enzymatic pathway of Cupriavidus basilensis strain DSM 22875.

---


Process for the production of second-generation biofuels from a lignocellulose-containing material comprising lignocellulose hydrolysate with a host microorganism expressing enzymes which convert furan compounds to non-toxic components, and conversion of the fermentable sugars from the lignocellulose hydrolysate or the detoxified lignocelluloses hydrolysate to produce a biofuel.

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<table>
<thead>
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<th>Patent document cited in search report</th>
<th>Publication date</th>
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<tr>
<td>US 7067303</td>
<td>B1</td>
<td>27-06-2006</td>
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