



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification <sup>7</sup> : <b>C12N 15/52, 15/53, 9/00, 9/02, 9/06, 1/19, C12P 7/06, 7/20 // (C12N 1/19, C12R 1:865)</b></p>	<b>A3</b>	<p>(11) International Publication Number: <b>WO 00/03021</b></p> <p>(43) International Publication Date: 20 January 2000 (20.01.00)</p>																																																																																																																																																																
<p>(21) International Application Number: PCT/DK99/00398</p> <p>(22) International Filing Date: 12 July 1999 (12.07.99)</p> <p>(30) Priority Data: PA 1998 00968 10 July 1998 (10.07.98) DK</p> <p>(71)(72) Applicants and Inventors: NIELSEN, Jens [DK/DK]; Hans Bruuns Vej 8, DK-2920 Charlottenlund (DK). NISSEN, Torben, Lauesgaard [DK/DK]; Schlegels Allé 8, 2, tv., DK-1807 Frederiksberg C (DK).</p> <p>(72) Inventor; and (75) Inventor/Applicant (for US only): KIELLAND-BRANDT, Morten, C. [DK/DK]; Lundbyesgade 3, DK-1771 Copenhagen V (DK).</p> <p>(74) Agent: BUDDE, SCHOU &amp; OSTENFELD A/S; P.O. Box 1183, Bredgade 4l, DK-1011 Copenhagen K (DK).</p>	<p>(81) Designated States: AE, AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL (Utility model), TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b> <i>With international search report.</i></p> <p>(88) Date of publication of the international search report: 20 April 2000 (20.04.00)</p>																																																																																																																																																																	
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<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p style="text-align: center; margin-bottom: 5px;">84.7 % Identity</p> <table style="width: 100%; border-collapse: collapse; font-family: monospace; font-size: 0.8em;"> <tr> <td style="width: 5%; text-align: right;">5</td> <td style="width: 5%;"></td> <td style="width: 5%;"></td> <td style="width: 87%;"></td> </tr> <tr> <td style="text-align: right;">A. vinelandii</td> <td style="text-align: right;">1</td> <td style="text-align: right;">1</td> <td>MAYNYDQVYIGTFCAGDANNAYAGKRVAVVDDRPVQGNCTHLEGTI</td> </tr> <tr> <td style="text-align: right;">P. fluorescens</td> <td style="text-align: right;">1</td> <td style="text-align: right;">1</td> <td>MAYNTDQVYVLSGSPAGEGAMMAKAGKRVAVVDDRPVQGNCTHLEGTI</td> </tr> <tr> <td colspan="4" style="text-align: center;">*****</td> </tr> <tr> <td style="text-align: right;">10</td> <td></td> <td></td> <td></td> </tr> <tr> <td style="text-align: right;">A. vinelandii</td> <td style="text-align: right;">51</td> <td style="text-align: right;">51</td> <td>PSKALAHSVRQIQYNNPFLFRQIGEPFRMFSFADVLSAEQVIAQVSSR</td> </tr> <tr> <td style="text-align: right;">P. fluorescens</td> <td style="text-align: right;">51</td> <td style="text-align: right;">51</td> <td>PSKALAHSVRQIQYNNPFLFRQIGEPFRMFSFADVLSAEQVIAQVSSR</td> </tr> <tr> <td colspan="4" style="text-align: center;">*****</td> </tr> <tr> <td style="text-align: right;">15</td> <td></td> <td></td> <td></td> </tr> <tr> <td style="text-align: right;">A. vinelandii</td> <td style="text-align: right;">101</td> <td style="text-align: right;">101</td> <td>TCYARHRIDTFYGTASFCDEHTIEVVRHAGNVETLVAQEVVIATSRFY</td> </tr> <tr> <td style="text-align: right;">P. fluorescens</td> <td style="text-align: right;">101</td> <td style="text-align: right;">101</td> <td>TCYARHRIDTFYGTASFCDEHTIEVVRHAGNVETLVAQEVVIATSRFY</td> </tr> <tr> <td colspan="4" style="text-align: center;">*****</td> </tr> <tr> <td style="text-align: right;">20</td> <td></td> <td></td> <td></td> </tr> <tr> <td style="text-align: right;">A. vinelandii</td> <td style="text-align: right;">151</td> <td style="text-align: right;">151</td> <td>RPADVDFTHFRIDYSDTILSLGHTPARKLIYGAGVIGCEYASIFSGLEVL</td> </tr> <tr> <td style="text-align: right;">P. fluorescens</td> <td style="text-align: right;">151</td> <td style="text-align: right;">151</td> <td>RPADIDFTHFRIDYSDTILSLGHTPARKLIYGAGVIGCEYASIFSGLEVL</td> </tr> <tr> <td colspan="4" style="text-align: center;">*****</td> </tr> <tr> <td style="text-align: right;">25</td> <td></td> <td></td> <td></td> </tr> <tr> <td style="text-align: right;">A. vinelandii</td> <td style="text-align: right;">201</td> <td style="text-align: right;">201</td> <td>VGLDNRDQLLSFLDDEISDLSYHLRNNHVLRRHNEEYERVELDNGVI</td> </tr> <tr> <td style="text-align: right;">P. fluorescens</td> <td style="text-align: right;">201</td> <td style="text-align: right;">201</td> <td>VGLVNRDQLLSFLDDEISDLSYHLRNNHVLRRHNEEYERVELDNGVI</td> </tr> <tr> <td colspan="4" style="text-align: center;">*****</td> </tr> </table> </div> <div style="width: 48%;"> <table style="width: 100%; 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<p>(57) Abstract</p> <p>The present invention concerns a microbial cell comprising a first expressible enzyme activity, e.g., pyridine nucleotide transhydrogenase from <i>Azotobacter vinelandii</i> which, when expressed in said microbial cell, is controlling an intracellular redox system of said cell, said first expressible enzyme activity in said microbial cell being operably linked to an expression signal not natively associated with said first expressible enzyme activity. The expression of said first expressible enzyme activity is operably linked to an increased production of a first metabolite. The cell is preferably a yeast cell and may also comprise a further expressible enzyme activity, e.g., glutamate synthase and/or glutamine synthetase, said further expressible enzyme activity, when expressed, mediating a first biosynthetic reaction resulting in production of a first metabolite, said further expressible enzyme activity, when expressed at an increased level, resulting in an increased production of said first metabolite, said increased expression of said further expressible enzyme activity and/or said increased production of said first metabolite being operably linked to an increased expression of said first expressible enzyme activity. There is also provided a microbial cell wherein said expression of said first expressible enzyme activity is operably linked to an increased production of a first metabolite and a decreased production of a second metabolite. Cells according to the invention are useful in the production of a metabolite such as ethanol or glycerol.</p>																																																																																																																																																																		

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EE	Estonia	LR	Liberia	SG	Singapore		

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/DK 99/00398

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>			
IPC 7	C12N15/52 C12N1/19	C12N15/53 C12P7/06	C12N9/00 C12P7/20
			C12N9/02 C12N9/06 //(C12N1/19,C12R1:865)
According to International Patent Classification (IPC) or to both national classification and IPC			
<b>B. FIELDS SEARCHED</b>			
Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12N C12P			
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched			
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)			
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>			
Category *	Citation of document, with indication, where appropriate, of the relevant passages		Relevant to claim No.
X	WO 98 18909 A (FRENCH CHRISTOPHER EDWARD ;UNIV CAMBRIDGE TECH (GB); BRUCE NEIL CH) 7 May 1998 (1998-05-07)		1,2,6, 8-12,14, 17-19, 28-30, 37,44, 45,47, 51,53, 54,56, 57, 64-66,70 13,35
Y	Note: 82.2% nt sequence identity of SEQ ID NO:1 with SEQ ID NO:1 in 1395 nt overlap, 84.1% aa sequence identity of SEQ ID NO:2 with SEQ ID NO:2 in 464 bp overlap. page 2, line 25 -page 3, line 5 page 3, line 7 -page 4, line 2 page 4, line 9-13 example 3 claims 1-8  -/--		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <span style="margin-left: 200px;"><input checked="" type="checkbox"/> Patent family members are listed in annex.</span>			
* 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 or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family			
Date of the actual completion of the international search		Date of mailing of the international search report	
25 January 2000		11/02/2000	
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer  van de Kamp, M	

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Int: :ional Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p style="text-align: center;">---</p> VOORDOUW G ET AL: "Why are two different types of pyridine nucleotide transhydrogenase found in living organisms?" EUROPEAN JOURNAL OF BIOCHEMISTRY, DE, BERLIN, vol. 131, no. 3, April 1983 (1983-04), pages 527-533, XP000853531 ISSN: 0014-2956 cited in the application	31-34,36
Y	abstract page 527 page 528, left-hand column, paragraphs 2,3 page 528, right-hand column, paragraph 4 -page 529, left-hand column, paragraph 1 figure 1	13,35
X	<p style="text-align: center;">---</p> TANTIRUNGKIJ M ET AL: "Expression of Escherichia coli transhydrogenase genes in Saccharomyces cerevisiae" MICROB. UTIL. RENEWABLE RESOUR. (1996), VOLUME DATE 1995, vol. 9, pages 664-672, XP000866358  the whole document page 670, paragraph 3	1-12, 14-19, 28-30, 37,38, 41,42, 44-62, 64-68,70
X	<p style="text-align: center;">---</p> EP 0 733 712 A (AJINOMOTO KK) 25 September 1996 (1996-09-25) cited in the application  page 2, line 48-55 page 3, line 3-5,11-45 examples 1-4 claims 1-8	1,2,6, 8-12,19, 28-30, 37,44, 45, 47-49, 51,53, 54,56, 57, 64-66,70
X	<p style="text-align: center;">---</p> WO 96 41888 A (INST NAT RECH AGRONOMIQUE IN ;DEQUIN SYLVIE (FR); BARRE PIERRE (FR)) 27 December 1996 (1996-12-27)  the whole document example 2 claims 1,10	1-9,19, 28-30, 37,38, 42, 44-61, 65-68,70
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 99/00398

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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