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LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
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CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ASCORBIC ACID PRODUCTION FROM YEASTS

(57) **Abstract:** Herein is disclosed a method of generating ascorbic acid from yeast. In one embodiment, the yeast is a *Zygosaccharomyces* or a *Kluyveromyces* spp. cultured in a medium comprising an ascorbic acid precursor. In a second embodiment the yeast is a recombinant yeast growing in a medium comprising an ascorbic acid precursor. Preferably the recombinant yeast is transformed with a coding region encoding an enzyme selected from L-galactose dehydrogenase (LGDH), L-galactono-1,4-lactone dehydrogenase (AGD), D-arabinose dehydrogenase (ARA), D-arabinono-1,4-lactone oxidase (ALO), L-gulonono-1,4-lactone oxidase (GLO), and aldonolactonase (AL). The ascorbic acid precursor is preferably D-glucose, L-galactose, L-galactono-1,4-lactone, L-gulonono-1,4-lactone, or L-gulonic acid. In another preferred embodiment the ascorbic acid is accumulated in the medium at levels greater than background. Preferably, the yeast is capable of converting about 25 % of the ascorbic acid precursor to L-ascorbic acid.



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A. CLASSIFICATION OF SUBJECT MATTER

 IPC 7 C12P17/04 C12N1/19 C12N15/53 C12N15/55 //C12R1:865,
 C12R1:645

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12P C12N C12R

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)

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 00 34502 A (KUMAR MANOJ ; GENENCOR INT (US)) 15 June 2000 (2000-06-15) cited in the application claim 8; table 1	1-131
Y	HANCOCK ROBERT D ET AL: "Biosynthesis of L-ascorbic acid (vitamin C) by Saccharomyces cerevisiae." FEMS MICROBIOLOGY LETTERS, vol. 186, no. 2, 15 May 2000 (2000-05-15), pages 245-250, XP002192486 ISSN: 0378-1097 cited in the application the whole document	1-6, 129
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in 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 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

20 June 2002

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 01/03485

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ONOFRI S ET AL: "Influence of L-galactonic acid gamma-lactone on ascorbate production in some yeasts." ANTONIE VAN LEEUWENHOEK, vol. 71, no. 3, 1997, pages 277-280, XP002203000 ISSN: 0003-6072 the whole document	1-6, 129
X	WO 98 50558 A (BAUW GUY JEROME CORNEEL; VLAAMS INTERUNIV INST BIOTECH (BE); OSTER) 12 November 1998 (1998-11-12) example 4	53
Y	claim 21; example 4	7-14, 17-19, 24-50, 56, 63, 66, 71-76, 78, 86-93, 95-113, 117-124, 126-131
Y	LEE BYUNG-HOON ET AL: "Bacterial production of D-erythroascorbic acid and L-ascorbic acid through functional expression of Saccharomyces cerevisiae D-arabinono-1,4-lactone oxidase in Escherichia coli." APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 65, no. 10, October 1999 (1999-10), pages 4685-4687, XP002192487 ISSN: 0099-2240 cited in the application the whole document	7-15, 24-50, 54, 64, 67, 68, 71-76, 80, 83, 84, 87-93, 95-115, 120-124, 126-131

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INTERNATIONAL SEARCH REPORT

 International Application No.
 PCT/GB 01/03485

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUH W-K ET AL: "D-ERYTHROASCORBIC ACID IS AN IMPORTANT ANTIOXIDANT MOLECULE IN SACCHAROMYCES CEREVISIAE" MOLECULAR MICROBIOLOGY, BLACKWELL SCIENTIFIC, OXFORD, GB, vol. 30, no. 4, 1998, pages 895-903, XP000870042 ISSN: 0950-382X cited in the application	54
Y	page 897, column 2 -page 899, column 1	7-15, 24-50, 57,58, 64,67, 68, 71-76, 80,83, 84, 87-93, 95-115, 120-124, 126-131
Y	WO 99 64618 A (DCV INC) 16 December 1999 (1999-12-16) cited in the application	7-14, 16-19, 24-51, 53,56, 57,61, 63,66, 67, 71-78, 83, 86-93, 95-114, 117-124, 126-131
	page 9, line 9 - line 18; claims 1,50,51	
Y	KIM S-T ET AL: "D-Arabinose dehydrogenase and its gene from Saccharomyces cerevisiae" BIOCHIMICA ET BIOPHYSICA ACTA. PROTEIN STRUCTURE AND MOLECULAR ENZYMOLOGY, ELSEVIER, AMSTERDAM,, NL, vol. 1429, no. 1, 8 December 1998 (1998-12-08), pages 29-39, XP004278563 ISSN: 0167-4838 cited in the application the whole document	7-14, 22-50, 52,58, 62,68, 71-76, 79,84, 87-113, 115, 120-131
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 01/03485

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>KRASNOV ALEKSEI ET AL: "Expression of rat gene for L-gulono-gamma-lactone oxidase, the key enzyme of L-ascorbic acid biosynthesis, in guinea pig cells and in teleost fish rainbow trout (<i>Oncorhynchus mykiss</i>)."</p> <p>BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1381, no. 2, 23 July 1998 (1998-07-23), pages 241-248, XP002192489 ISSN: 0006-3002 the whole document</p>	<p>7-14, 20, 24-50, 55, 60, 65, 70-76, 81, 85, 87-93, 95-113, 116, 120-124, 126-131</p>
Y	<p>KANAGASUNDARAM V ET AL: "ISOLATION AND CHARACTERIZATION OF THE GENE ENCODING GLUCONOLACTONASE FROM <i>ZYMONAS MOBILIS</i>"</p> <p>BIOCHIMICA ET BIOPHYSICA ACTA, AMSTERDAM, NL, vol. 1171, no. 2, 1992, pages 198-200, XP000901575 ISSN: 0006-3002 the whole document</p>	<p>7-14, 21, 24-50, 59, 60, 69-76, 82, 85, 87-93, 95-113, 116, 120-124, 126-131</p>
A	<p>WO 99 33995 A (SMIRNOFF NICK ; ASCORBEX LTD (GB); WHEELER GLEN (GB); BIOTECHNOLOGY) 8 July 1999 (1999-07-08) cited in the application page 15 -page 18</p>	<p>1-131</p>

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Box I Observations where certain claims were found unsearchable (Continuation of item 1 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 II Observations where unity of invention is lacking (Continuation of item 2 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 fee, this Authority did not invite payment of any additional fee.
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.:
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.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: Claims: 1-6, 129 (all partially)

method for the production of ascorbic acid by the cultivation of a *Kluyveromyces* spp. yeast in a medium containing an ascorbic acid precursor

Invention 2: Claims 1-6, 129 (all partially)

method for the production of ascorbic acid by the cultivation of a *Zygosaccharomyces* spp. yeast in a medium containing an ascorbic acid precursor

Invention 3: Claims 7-14, 24-50, 71-76, 87-93, 95-113, 120-128, 130, 131 (all partially), 16, 51, 61, 77 (all completely)

method for the production of ascorbic acid by the cultivation of a yeast containing a heterologous gene encoding L-galactose dehydrogenase in a medium containing an ascorbic acid precursor

Invention 4: Claims 7-14, 24-50, 71-76, 87-93, 95-113, 120-128, 130, 131 (all partially), 22, 23, 52, 62, 79, 94 (all completely)

method for the production of ascorbic acid by the cultivation of a yeast containing a heterologous gene encoding D-arabinose dehydrogenase in a medium containing an ascorbic acid precursor

Invention 5: Claims 7-14, 24-50, 71-76, 87-93, 95-113, 120-128, 130, 131 (all partially), 17-19, 53, 56, 63, 66, 78, 86, 117-119 (all completely)

method for the production of ascorbic acid by the cultivation of a yeast containing a heterologous gene encoding L-galactono-1,4-lactone dehydrogenase in a medium containing an ascorbic acid precursor

Invention 6: Claims 7-14, 24-50, 71-76, 87-93, 95-113, 120-128, 130, 131 (all partially), 15, 54, 57, 58, 64, 67, 68, 80, 83, 84, 114, 115 (all completely)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

method for the production of ascorbic acid by the cultivation of a yeast containing a heterologous gene encoding D-arabino-1,4-lactone oxidase in a medium containing an ascorbic acid precursor

Invention 7: Claims 7-14, 24-50, 71-76, 87-93, 95-113, 120-128, 130, 131 (all partially), 20, 55, 65, 81, 85, 116 (all completely)

method for the production of ascorbic acid by the cultivation of a yeast containing a heterologous gene encoding L-gulono-1,4-lactone oxidase in a medium containing an ascorbic acid precursor

Invention 8: Claims 7-14, 24-50, 71-76, 87-93, 95-113, 120-128, 130, 131 (all partially), 21, 59, 60, 69, 70, 82 (all completely)

method for the production of ascorbic acid by the cultivation of a yeast containing a heterologous gene encoding aldonolactonase in a medium containing an ascorbic acid precursor

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/GB 01/03485

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