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(54) Title: PROMOTERS DERIVED FROM YARROWIA LIPOLYTICA AND ARXULA ADENINIVORANS, AND METHODS OF USE THEREOF

(57) Abstract: Disclosed are the nucleotide sequences of promoters from Arxula adeninivorans and Yarrowia lipolytica which may be used to drive gene expression in a cell. The promoters were validated, and selected promoters were screened to determine which promoters may be -useful for increasing the lipid production efficiency of oleaginous yeasts.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US15/41910

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - C12N 1/19, C12N 15/81, 15/90 (2015.01) CPC - C12N 15/81, 15/1093, 15/52 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(8) Classification(s): C12N1/19, C12N15/81, C12N15/90, C12N15/52 (2015.01) CPC Classification(s): C12N15/81, C12N15/1093, C12N15/52, C12N15/905 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google/Google Scholar; EBSCO; PubMed; 'Arxula adenivorans', 'Translation Elongation factor EF-1alpha', TEF1, 'nucleic acid', 'parent cell', promoter		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y Y A	EP 1698702 A1 (GELLISSSEN G) June 09, 2006; abstract; Claims 1-16 KUNZE, G. Characterization Of Arxula Adenivorans Strains From Different Habitats: Arxula Adenivorans TEF Gene For Translation Elongation Factor EF-1-alpha: GenBank: Z47379.1: Direct Submission. Antonie Van Leeuwenhoek. Vol. 65, No. 1; 06 January 1995; pages 29-34. US 2008/0118950 A1 (GELLISSSEN, G et al) May 22, 2008; abstract	1, 2 ----- 3-4, 10, 17 3-4, 10, 17 1-5, 10, 17
<input type="checkbox"/> Further documents are listed in the continuation of Box C.		
<input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 14 December 2015 (14.12.2015)		Date of mailing of the international search report 12 JAN 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300		Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US15/41910

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.: 6-9,11-16, 18-25
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:

-***-Please See Supplemental Page-***-

- 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 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.:

- 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.:

Groups I+: Claims 1-5, 10, 17, SEQ ID NO: 5 (Arxula adenivorans DNA sequence)

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.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US15/41910

-***-Continuation of Box No. III: Observations Where Unity Of Invention Is Lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+: Claims 1-5, 10 and 17 are directed toward nucleic acid encoding a promoter from *Arxula adenivorans*; a transformed cell comprising a genetic modification with said promoter; and a method for expressing a gene in a cell, comprising transforming a cell with a nucleic acid comprising said promoter.

The nucleic acid, transformed cell, and method for expressing a gene will be searched to the extent that the nucleic acid encompasses SEQ ID NO: 5 (*Arxula adenivorans* DNA sequence). It is believed that Claims 1, 2, 3 (in-part), 4 (in-part), 5 (in-part), 10 (in-part) and 17 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 5 (*Arxula adenivorans* DNA sequence). Applicant is invited to elect additional promoter(s), with specified SEQ ID NO: for each, to be searched. Additional promoter sequence(s) will be searched upon the payment of additional fees. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An Exemplary Election would be: SEQ ID NO: 6 (*Arxula adenivorans* DNA sequence).

No technical features are shared between the promoter sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a nucleic acid encoding a promoter from *Arxula adenivorans*, wherein the promoter is a promoter for Translation Elongation factor EF-1alpha; Glycerol-3-phosphate dehydrogenase; Triosephosphate isomerase 1; Fructose-1,6-bisphosphate aldolase; Phosphoglycerate mutase; Pyruvate kinase; Export protein EXP1; Ribosomal protein S7; Alcohol dehydrogenase; Phosphoglycerate kinase; Hexose transporter; General amino acid permease; Serine protease; Isocitrate lyase; Acyl-CoA oxidase; ATP-sulfurylase; Hexokinase; 3-phosphoglycerate dehydrogenase; Pyruvate Dehydrogenase Alpha subunit; Pyruvate Dehydrogenase Beta subunit; Aconitase; Enolase; Actin; Multidrug resistance protein (ABC-transporter); Ubiquitin; GTPase; Plasma membrane Na⁺/Pi cotransporter; Pyruvate decarboxylase; Phytase or Alpha-amylase; a transformed cell, comprising a genetic modification, wherein said genetic modification is transformation with a nucleic acid encoding a promoter, wherein the promoter has at least 90% sequence homology with a subsequence of any of SEQ ID NOs: 5-53, and said subsequence retains promoter activity; and a method for expressing a gene in a cell, comprising transforming a parent cell with a nucleic acid encoding a promoter, wherein the promoter has at least 90% sequence homology with a subsequence of any of SEQ ID NOs: 5-53; said subsequence retains promoter activity; and either the nucleic acid comprises the gene, and the gene and the promoter are operably linked; or the nucleic acid is designed so that the promoter becomes operably linked to the gene after transformation of the parent cell.

However, these shared technical features are previously disclosed by US 2008/0118950 A1 to Gellissen, et al. (hereinafter 'Gellissen') in view of the publication entitled 'Characterization Of *Arxula adenivorans* Strains From Different Habitats: *Arxula adenivorans* TEF Gene For Translation Elongation Factor EF-1-alpha: GenBank: Z47379.1' by Kunze, et al. (hereinafter 'Kunze').

Gellissen discloses a nucleic acid (an expression plasmid (a nucleic acid); paragraph [0022]) encoding a promoter from *Arxula adenivorans* (encoding a promoter from *Arxula adenivorans*; paragraph [0022]), wherein the promoter is a promoter for Translation Elongation factor EF-1alpha (wherein the promoter is a TEF1 promoter (for Translation Elongation factor EF-1alpha); paragraph [0022]); a transformed cell (a transformed cell; paragraphs [0005], [0010]), comprising a genetic modification (comprising an additional recombinant DNA sequence (comprising a genetic modification); paragraph [0010]), wherein said genetic modification is transformation (wherein said genetic modification is transformation; paragraph [0010]) with a nucleic acid encoding a promoter (with a plasmid comprising a promoter (with a nucleic acid encoding a promoter); paragraph [0022]), wherein the promoter is an *Arxula adenivorans* TEF1 promoter (wherein the promoter is an *Arxula adenivorans* TEF1 promoter; paragraph [0022]); and a method for expressing a gene in a cell (a method for expressing a gene in a cell; abstract), comprising transforming a parent cell (comprising transforming a parent cell; paragraph [0010]) with a nucleic acid encoding a promoter (with a plasmid comprising a promoter (with a nucleic acid encoding a promoter); paragraph [0022]), wherein the promoter is an *Arxula adenivorans* TEF1 promoter (wherein the promoter is an *Arxula adenivorans* TEF1 promoter; paragraph [0022]); and the gene and the promoter are operably linked (and expression of the gene is controlled by the promoter (the gene and the promoter are operably linked); paragraph [0022]). Gellissen does not disclose wherein the promoter has at least 90% sequence homology with a subsequence of SEQ ID NO: 5, wherein the subsequence has promoter activity. Kunze discloses the *Arxula adenivorans* TEF gene for translation elongation factor EF-1 alpha (the *Arxula adenivorans* TEF gene for translation elongation factor EF-1 alpha; page 1), and a subsequence having 92.9% homology with a subsequence of SEQ ID NO: 5 (a nucleic acid sequence encoding EF-1alpha (a subsequence having 92.9% homology with a subsequence of SEQ ID NO: 5 of the instant PCT application (wherein nt 1-292 of the nucleic acid sequence disclosed are 92.9% identical to nt 124-415 of SEQ ID NO: 5 of the instant PCT application; wherein, as the indicated nucleic acid sequence ORF begins at nt 305, the sequence having homology with SEQ ID NO: 5 of the instant PCT application has promoter activity based on its proximity to the beginning of the translated product). It would have been obvious to a person of ordinary skill in the art, at the time the invention, to have modified the previous disclosure of Gellissen, for utilizing the TEF gene, and its promoter region, as previously disclosed by Kunze, for providing the necessary promoter for the expression plasmid, as previously disclosed by Gellissen, and, by providing the sequence thereof, for enabling a person of ordinary skill in the art, at the time the invention, to reproduce the expression plasmids of Gellissen, or variants thereof, through routine experimentation and testing.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Gellissen and Kunze references, unity of invention is lacking.