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(54) Title: EXPRESSION OF MODIFIED PROTEINS IN A PEROXISOME

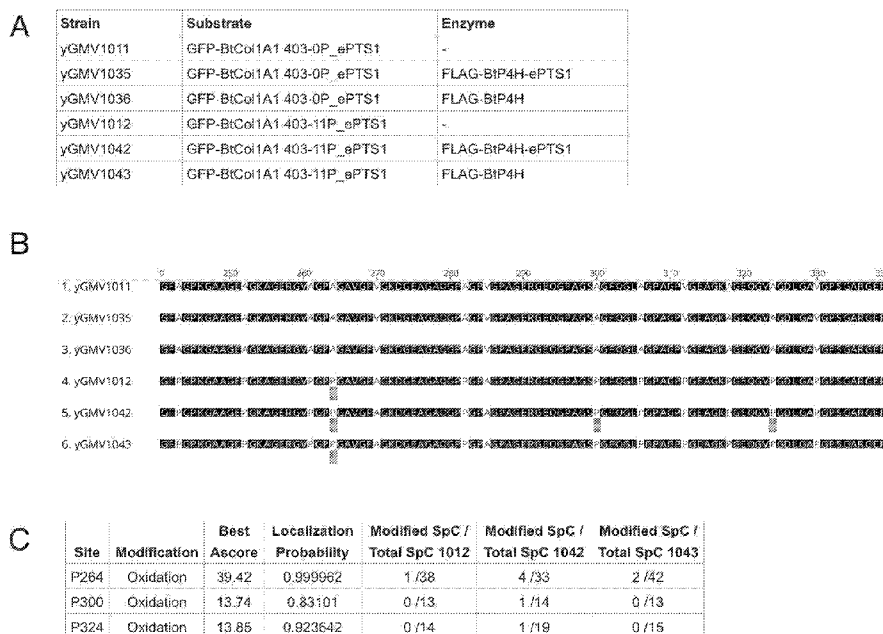


FIG. 7

(57) Abstract: Disclosed herein include methods and compositions for making proteins in peroxisomes as well as methods of making cells for producing proteins in peroxisomes. Also disclosed herein are cells for producing a protein in a peroxisome, and methods for producing a protein in a eukaryotic cell containing a peroxisome as described herein.



UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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- *with sequence listing part of description (Rule 5.2(a))*

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/32512

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 9/02; C12N 15/81; C07K 14/78; C07K 19/00 (2020.01)

CPC - C12N 15/81; C07K 14/78; C12N 9/0004; C07K 2319/01

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)

See Search History document

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

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WANG et al. Targeting expression of the catalytic domain of the kinase insert domain receptor (KDR) in the peroxisomes of <i>Pichia pastoris</i> . FEMS Yeast Res. August 2009, Vol 9, No 5, Pages 732-741. Especially abstract, (pg 735 col 2 para 3.	1-3, 37-41
Y	US 2002/0142391 A1 (KIVIRIKKO et al.) 3 October 2002 (03.10.2002). Especially para [0099], [0169], claims 1, 2, 5	1-3, 37-41

☐ 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

"D" document cited by the applicant in the international application

"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

21 October 2020

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/32512

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.: 4-29, 33-36
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:

-----Go to Extra Sheet for continuation-----

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.:
Claims 1-3, 37-41

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/US 20/32512

Continuation of Box III: Observation 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 searched, the appropriate additional search fees must be paid.

Group I: Claims 1-3, 37-41, drawn to a method of producing a modified protein in a peroxisome.

Group II: Claims 30-32, 42, drawn to a composition comprising a eukaryotic cell for producing a modified protein in a peroxisome.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I has the special technical feature of specific method steps for producing a modified protein in a peroxisome, not required by Group II.

Group II has the special technical feature of a eukaryotic cell composition for producing a modified protein in a peroxisome, not required by Group I.

Common Technical Feature:

Groups I and II share the common technical feature of a eukaryotic cell for producing a protein in a peroxisome, the cell comprising: a first nucleic acid comprising a sequence encoding a heterologous protein fused to a peroxisome-targeting sequence; and a second nucleic acid encoding a heterologous modification enzyme fused to a peroxisome-targeting sequence

However, said common technical feature does not represent a contribution over the prior art, and is disclosed by the publication titled "Targeting expression of the catalytic domain of the kinase insert domain receptor (KDR) in the peroxisomes of *Pichia pastoris*" by Wang et al. (hereinafter "Wang") [published in FEMS Yeast Res, August 2009, Vol 9, No 5, Pages 732-741], in view of US 2005/0164345 A1 to Kivirikko et al. (hereinafter "Kivirikko").

As to the common technical feature, Wang discloses a eukaryotic cell for producing a protein in a peroxisome, the cell comprising: a first nucleic acid comprising a sequence encoding a heterologous protein fused to a peroxisome-targeting sequence (abstract; "we expressed the catalytic domain of KDR in *Pichia pastoris* under the control of the AOX1 promoter. In order to facilitate its purification and detection, His-tag and green fluorescent protein (GFP) were fused to the N-terminus of KDR. At the same time, a peroxisomal targeting signal 1 (SKL) was fused to the C-terminus to avoid the potential negative effect on the host cell. The highly expressing clone K1 was selected by GFP fluorescence"); and a second nucleic acid comprising a sequence encoding a heterologous protein fused to a peroxisome-targeting sequence (pg 735 col 2 para 3; "Clone K1 expressing the GFP-KDR-SKL fusion protein was transformed with plasmid pRDM054, which expressed the blue fluorescent protein (BFP)-SKL [peroxisomal targeting signal 1, SKL] under the control of the AOX1 promoter"; abstract; "Furthermore, the GFP-KDR-SKL fusion protein was proved to be correctly targeted to the peroxisomes of *P. pastoris* by colocalization with blue fluorescent protein-SKL").

Wang does not disclose that the second nucleic acid encodes a heterologous modification enzyme that is capable of modifying the protein product of the first nucleic acid.

However, strategies of co-expressing, in a eukaryotic host cell, a first recombinant protein and a second recombinant protein, where the second recombinant protein is an enzyme capable of modifying the first recombinant protein was well-known in the art, as disclosed by Kivirikko (para [0033]; "The present invention comprises the expression of at least one nucleic acid sequence encoding a collagen chain, and at least one nucleic acid sequence encoding a collagen post-translational enzyme"; para [0160]; "Expression of Recombinant Collagen Genes in *Saccharomyces cerevisiae* Yeast Expressing Recombinant Genes for Prolyl 4-Hydroxylase"; para [0026]; "Prolyl 4-hydroxylase is an important post-translational enzyme necessary for the synthesis of procollagen or collagen by cells. The enzyme is required to hydroxylate prolyl residues in the Y-position of the repeating -Gly-X-Y- sequences to 4-hydroxyproline").

An artisan of ordinary skill in the art would have recognized that the strategy disclosed by Wang, where both the first and second nucleic acids encoding protein could be localized to the peroxisome because both proteins have a fusion peroxisomal targeting signal could have been applied to a first recombinant protein and a second recombinant protein, where the second recombinant protein is an enzyme capable of modifying the first recombinant protein, as disclosed by Kivirikko, without undue effort and a high probability of success.

As the common technical feature was known in the art at the time of the invention, this cannot be considered a common special technical feature that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I and II lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Item 4 (cont.): Claims 4-29, 33-36 are held unsearchable because they are not drafted according to the second and third sentences of PCT Rule 6.4(a).