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(54) Title: ENZYMATIC PRODUCTION OF ALLULOSE

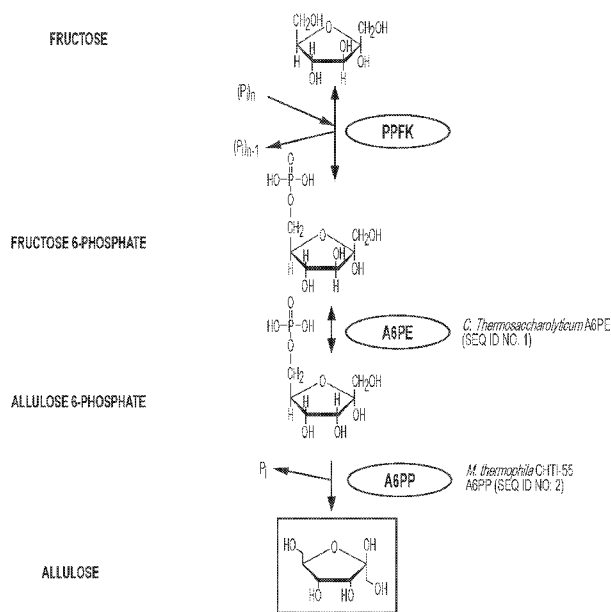


FIG. 3

(57) Abstract: The invention relates to improved processes for the enzymatic production of allulose using enzymes which have been characterized as having improved expression, improved stability, and low allulose to fructose conversion activity, relative to enzymes in other allulose production methods. Improved processes include steps of converting fructose-6-phosphate to allulose 6-phosphate (A6P) using an allulose 6-phosphate epimerase, and converting A6P to allulose using an allulose-6-phosphate phosphatase.



TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

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- *with international search report (Art. 21(3))*
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/32952

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12P 19/24, C12P 19/00, C12P 19/02, C12N 9/16, C12N 9/90, C12N 9/88 (2021.01)

CPC - C12Y 207/01002, C12N 9/1205

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	TW 2019/43854 A (BONUMOSE LLC) 16 November 2019 (16.11.2019) Abstract; Claim 2; Claim 3; Claim 4; Claim 5; Claim 6; Claim 7; Claim 8; Claim 9; Claim 10; Claim 11; Claim 13	1, 4-13
Y	UniProtKB submission A0A223HZI7, Allulose-6-phosphate 3-epimerase, 25 October 2017 [online]. [Retrieved on 16 September 2021]. Retrieved from the internet: <URL: https://www.uniprot.org/uniprot/A0A223HZI7 > Entire document	1, 4-13



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

16 September 2021

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/32952

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.: 17-20, 22-23
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 continuation in first extra 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 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.:
1, (4-13)(in part), limited to the process of converting F6P to A6P using an A6PE enzyme of SEQ ID NO:1

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

International application No.

PCT/US 21/32952

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 searched, the appropriate additional search fees must be paid.

Group I+, Claims 1-16, 21, directed to an improved process for the production of allulose from a saccharide. The process will be searched to the extent that the process encompasses an improvement comprising converting fructose-6-phosphate (F6P) to allulose 6-phosphate (A6P) using an allulose 6-phosphate epimerase (A6PE), wherein the A6PE comprises an amino acid sequence having at least 100% sequence identity to SEQ ID NO: 1 (note, this is the first claimed sequence for the inventive enzyme for the improved process embodiment). It is believed that claims 1, (4-13)(in part) encompass first named invention (because the inventive improvement is restricted to the process step of claim 1), and thus these claims will be searched without fee to the extent that the process encompasses an improvement comprising converting fructose-6-phosphate (F6P) to allulose 6-phosphate (A6P) using an allulose 6-phosphate epimerase (A6PE), wherein the A6PE comprises an amino acid sequence having at least 100% sequence identity to SEQ ID NO: 1. Additional process steps comprising additional enzyme(s) and additional upstream and/or downstream process step(s) mediated by additional enzyme(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected process steps comprising additional enzyme(s) and additional upstream and/or downstream process step(s) mediated by additional enzyme(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. 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. An exemplary election would be an improved process for the production of allulose from a saccharide, the improvement comprising converting A6P to allulose using an allulose-6-phosphate phosphatase (A6PP), wherein the A6PP comprises an amino acid sequence having at least 100% sequence identity to SEQ ID NO: 2 (claims 2-3, (4-13)(in part), 14-16, 21).

The inventions listed as Group I+ do not relate to a single special technical feature 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

The inventions of Group I+ each include the special technical feature of a unique amino acid sequence. Each amino acid sequence comprises a unique peptide, and is considered a distinct technical feature.

Common technical features

No technical features are shared between the enzyme amino acid sequences of Group I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Group I+ were considered to share the technical features of including:

an improved process for the production of allulose from a saccharide, the improvement comprising converting fructose-6-phosphate (F6P) to allulose 6-phosphate (A6P) using an allulose 6 phosphate epimerase (A6PE), wherein the A6PE comprises an amino acid sequence;

an improved process for the production of allulose from a saccharide, the improvement comprising converting A6P to allulose using an allulose-6-phosphate phosphatase (A6PP), wherein the A6PP comprises an amino acid sequence;

these shared technical features are previously disclosed by TW 2019/43854 A to Bonumose LLC, (hereinafter 'Bonumose').

Bonumose teaches an improved process for the production of allulose from a saccharide, the improvement comprising converting fructose-6-phosphate (F6P) to allulose 6-phosphate (A6P) using an allulose 6 phosphate epimerase (A6PE), wherein the A6PE comprises an amino acid sequence; and an improved process for the production of allulose from a saccharide, the improvement comprising converting A6P to allulose using an allulose-6-phosphate phosphatase (A6PP), wherein the A6PP comprises an amino acid sequence (Abstract - English translation 'The current disclosure provides a process for enzymatically converting a saccharide into allulose. The invention also relates to a process for preparing allulose where the process involves converting fructose 6-phosphate (F6P) to allulose 6-phosphate (A6P), catalyzed by allulose 6-phosphate 3-epimerase (A6PE), and converting the A6P to allulose, catalyzed by allulose 6-phosphate phosphatase (A6PP).'; Claim 13 - English translation 'The method according to item 12 of the patent application, wherein the allulose 6-phosphate 3-epimerase comprises ... at least 100% sequence identity amino acid sequences, wherein the epimerase Catalyzes the conversion of F6P to A6P.').

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+ inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

*Continuation of Item 4 above: claims 17-20, 22-23 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).