Title: PROCESS AND MATERIALS FOR PRODUCTION OF GLUCOSAMINE AND N-ACETYLGалУОСАМINE

Abstract: A biosynthetic method for producing glucosamine and N-acetylgalactosamine is disclosed. Such a method includes the fermentation of a genetically modified microorganism to produce glucosamine and/or N-acetylgalactosamine. Also disclosed are genetically modified microorganisms that are useful for producing glucosamine and N-acetylgalactosamine. In addition, methods of recovering N-acetylgalactosamine that have been produced by a fermentation process, including methods that result in N-acetylgalactosamine of high purity, are described. Also disclosed is a method to produce glucosamine from N-acetylgalactosamine.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8): C12N 8/10(2006.01), 1/20(2006.01); C12P 19/26(2006.01); C12Q 1/48(2006.01)
C12N 9/10(2006.01), 9/60(2006.01), 15/00(2006.01); C12P 21/06(2006.01); C07K 14/00(2006.01); C07H 21/04(2006.01)

USPC: 435/15, 193, 183, 325, 252, 252, 3, 320.1, 184; 530/350; 536/23.2

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)
U.S.: 435/15, 193, 183, 325, 252, 252, 252, 3, 320.1, 184; 530/350; 536/23.2

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)
MEDLINE, CAPLUS, EMBASE, BIOSIS, GENBANK, AGRICOLA, EMBASE, WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

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
  "B" earlier application or patent 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
  "I" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principles 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: 29 April 2007 (29.04.2007)

Date of mailing of the international search report: 23 May 2007

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Form PCT/ISA/210 (second sheet) (July 1998)
INTERNATIONAL SEARCH REPORT

Box I  Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim 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. ☐ Claim 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:
Please See Continuation 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.: 1-61,116-119 as they relate to SEQ ID NO:30

Remark on Protest ☐ The additional search fees were accompanied by the applicant’s protest.
☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)
BOX II. 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.

Group I, claim(s) 1-61, 116-119, drawn in part to an organism having a genetic modification that increases the activity of the glucosamine-6-phosphate acetyltransferase of SEQ ID NO: 30, and a method to produce glucosamine or N-acetylglucosamine by fermentation, wherein said method requires cultivation of said microorganism.

Group II, claim(s) 1-61, 116-119, drawn in part to an organism having a genetic modification that increases the activity of the glucosamine-6-phosphate acetyltransferase of SEQ ID NO: 32, and a method to produce glucosamine or N-acetylglucosamine by fermentation, wherein said method requires cultivation of said microorganism.

Group III, claim(s) 1-61, 116-119, drawn in part to an organism having a genetic modification that increases the activity of the glucosamine-6-phosphate acetyltransferase of SEQ ID NO: 34, and a method to produce glucosamine or N-acetylglucosamine by fermentation, wherein said method requires cultivation of said microorganism.

Group IV, claim(s) 62-89, drawn in part to a method to produce glucosamine or N-acetylglucosamine by fermentation, wherein said method requires cultivation of a microorganism that comprises a genetic modification that increases the activity of glucosamine-6-phosphate deaminase and the activity of the glucosamine-6-phosphate acetyltransferase of SEQ ID NO: 30.

Group V, claim(s) 62-89, drawn in part to a method to produce glucosamine or N-acetylglucosamine by fermentation, wherein said method requires cultivation of a microorganism that comprises a genetic modification that increases the activity of glucosamine-6-phosphate deaminase and the activity of the glucosamine-6-phosphate acetyltransferase of SEQ ID NO: 32.

Group VI, claim(s) 62-89, drawn in part to a method to produce glucosamine or N-acetylglucosamine by fermentation, wherein said method requires cultivation of a microorganism that comprises a genetic modification that increases the activity of glucosamine-6-phosphate deaminase and the activity of the glucosamine-6-phosphate acetyltransferase of SEQ ID NO: 34.

Group VII, claim(s) 90-107, drawn to a method to produce glucosamine or N-acetylglucosamine by fermentation, wherein said method requires cultivation of a microorganism that comprises a genetic modification that decreases the activity of glucosamine-1-phosphate N-acetyltransferase.

Group VIII, claim(s), 108-115, drawn to a method to produce glucosamine or N-acetylglucosamine by fermentation, wherein said method requires cultivation of a microorganism that comprises the endogenous glucosamine-6-phosphate acetyltransferase and a genetic modification that increases the activity of glucosamine-6-phosphate synthase.

Group IX, claim(s) 120-125, drawn to a genetically modified organism that comprises a genetic modification which increases the activity of glucosamine-6-phosphate deaminase.

Group X, claim(s) 126-127, drawn to a genetically modified organism that comprises a genetic modification which decreases the activity of glucosamine-6-phosphate deaminase and increases the activity of glucose-6-phosphate N-acetyltransferase.

Group XI, claim(s) 128-156, drawn to a method for recovering N-acetyltransferase from a fermentation broth.

Group XII, claim(s) 157-206, drawn to a method to produce glucosamine from a source of N-acetylglucosamine.

Group XIII, claim(s) 207-212, drawn to a method to produce glucosamine by fermentation, wherein said method requires cultivation of a microorganism that comprises a nucleic acid encoding a glucosamine-6-phosphate synthase, wherein the expression of said nucleic acid is controlled by a lactose induction.

The inventions listed as Groups I-XII 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:

Form PCT/ISA/210 (second sheet) (July 1998)
According to PCT Rule 13.2, unity of invention exists only when the shared same or corresponding special technical feature is a contribution over the prior art. The inventions listed as Groups I-XIII do not relate to a single general inventive concept because they lack the same or corresponding special technical feature. The technical feature linking Groups I-XIII is the production of glucosamine or N-acetylglucosamine by fermentation of microorganisms genetically altered such that the activities of enzymes involved in the synthesis of these compounds are either increased or decreased, which is shown by Berry et al. (U.S. Patent No. 6372457, issued 4/16/2002; cited in the specification) to lack novelty or inventive step since Berry et al. teach organisms genetically modified such that the activities of enzymes involved in the biosynthesis of glucosamine/N-acetylglucosamine are either increased/decreased to produce glucosamine (Examples 2-7 and 11). Thus, the technical feature does not make a contribution over the prior art and the claimed inventions do not meet the requirement of unity of invention under PCT Rule 13.2.

The organisms of Group IX and the methods of Groups IV-VI, or the organisms of Group X and the method of Group VII, while being combinations comprising a product and a process of use of said product, do not have unity of invention according to 37 CFR 1.475(b) since Group I (main invention) already contains one of the combinations set forth in 37 CFR 1.475(b), i.e., a product and a process of use of said product, and the combinations of Group IX and IV-VI, or Group X and VII are additional combinations as set forth in 37 CFR1.475(b)(2).