Title: PROMOTERS FROM CYANOBACTERIA ALLOWING HIGH EXPRESSION LEVELS

Abstract: The invention relates to the field of microbiology. More specifically, methods are provided for the identification of highly expressed genes and their corresponding promoters and UV responsive genes and their corresponding promoters in cyanobacteria Synechocystis sp. PCC6803. These genes and promoters can be used to construct expression vectors in cyanobacteria, green algae or plants, for the production of biomaterials from sunlight, a renewable energy resource.
INTERNATIONAL SEARCH REPORT

PCT/US 02/03926

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/77

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

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)

BIOSIS, EPO-Internal, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category* Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No.


PATENT ABSTRACTS OF JAPAN

vol. 1995, no. 06,
abstract

VICZIAN ANDRAS ET AL: "UV-B induced differential transcription of psbD genes encoding the D2 protein of Photosystem II in the cyanobacterium Synechocystis 6803."

PHOTOSYNTHESIS RESEARCH,
vol. 64, no. 2-3, 2000, pages 257-266,
X001085281
ISSN: 0166-8595

Date of the actual completion of the international search

16 July 2002

Date of mailing of the international search report

08.10.02

Name and mailing address of the ISA

European Patent Office, P.B. 5018 Patentlaan 2
NL - 2280 HV Rijswijk
Tel: (+31-70) 945-2040, Tx: 31 651 epo nl,
Fax: (+31-70) 945-3016

Authorized officer

Marinoni, J-C
<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>
<tbody>
<tr>
<td>A</td>
<td>ELHAI J: &quot;STRONG AND REGULATED PROMOTERS IN THE CYANOBACTERIUM ANABAENA PCC 7120&quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ISSN: 0378-1097</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>MATE ZOLTAN ET AL: &quot;UV-B-induced differential transcription of psbA genes encoding the D1 protein of photosystem II in the cyanobacterium Synechocystis 6803.&quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17439-17444, XP002206327</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ISSN: 0021-9258</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>WATANABE T ET AL: &quot;IDENTIFICATION OF 10SA RNA (TMRNA) HOMOLOGUES FROM THE CYANOBACTERIUM SYNECHOCYCCUS SP. STRAIN PCC6301 AND RELATED ORGANISMS&quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BIOCHIMICA ET BIOPHYSICA ACTA, AMSTERDAM, NL, vol. 1396, no. 1, 1998, pages 97-104,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>XP000949980</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ISSN: 0006-3002</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>KANEKO T ET AL: &quot;Sequence analysis of the genome of the unicellular cyanobacterium Synechocystis sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions&quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>XP002084893</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ISSN: 1340-2838</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>CHUNGJATUPORNCHAI W: &quot;EXPRESSION OF THE MOSQUITOCIDAL-PROTEIN GENES OF BACILLUS THURINGIENSIS SUBSP. ISRAELIENSIS AND THE HERBICIDE-RESISTANCE GENE BAR IN SYNECHOCYCTIS PCC6803&quot;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ISSN: 0343-8651</td>
<td></td>
</tr>
</tbody>
</table>
INTERNATIONAL SEARCH REPORT

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.: 1-16 all partially 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:
   see FURTHER INFORMATION sheet PCT/ISA/210

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:

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, 2, 7-26 all partially

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

1. Claims: 1,2,7-26 all partially

   Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the promoter of the amiC gene for expression during the log phase.

2. Claims: 1,2, 7-16 all partially

   Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the promoter of the rbcX gene for expression during the log phase.

3. Claims: 1,2,7-16 all partially

   Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the promoter of the gene having the sequence as set out in SEQ ID NO. 5 for expression during the log phase.

4. Claims: 3-16 all partially

   Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the hliB gene.

5. Claims: 3-16 all partially

   Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the hsp17 gene.

6. Claims: 3-16 all partially

   Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the nb1B gene.

7. Claims: 3-16 all partially

   Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the rpoD gene.
8. Claims: 3-16 all partially

Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the hliA gene.

9. Claims: 3-16 all partially

Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the ftsH gene.

10. Claims: 3-16 all partially

Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the clpB gene.

11. Claims: 3-16 all partially

Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the gene having the sequence as set out in SEQ ID No. 9.

12. Claims: 3-16 all partially

Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the gene having the sequence as set out in SEQ ID No. 11.

13. Claims: 3-16 all partially

Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the gene having the sequence as set out in SEQ ID No. 17.

14. Claims: 3-16 all partially

Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the gene having the sequence as set out in SEQ ID No. 21.

15. Claims: 3-16 all partially
Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the gene having the sequence as set out in SEQ ID No. 25.

16. Claims: 3-16 all partially

Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the gene having the sequence as set out in SEQ ID No. 27.

17. Claims: 3-16 all partially

Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the gene having the sequence as set out in SEQ ID No. 31.

18. Claims: 3-16 all partially

Methods for regulating the expression of gene of interest in Cyanobacteria, wherein the gene is under the control of the UV-B light inducible promoter of the gene having the sequence as set out in SEQ ID No. 39.
Continuation of Box I.2

Claims Nos.: 1-16 all partially

Claims 1-16 refer to methods wherein promoters are used. However, neither in the application nor in the prior art, are these promoters defined in terms of technical features (i.e. their sequence and their length). In the application as well as in the claims, the promoters are merely defined by reference to the sequence which expression they control. Moreover, the claims encompass methods for the production of proteins of interest in all cyanobacteria by using promoters of genes of undefined origin whereas the application relates only to promoters originating from Synechocystis sp. Even when considering only promoters of the cited Synechocystis as a restriction, and taking into consideration that the fact that the sequence of the Synechocystis genome is known (i.e. that the upstream sequences of the mentioned genes are somehow retrievable), one cannot define precisely what part (i.e. sequence and length) of said upstream sequences are intended to be covered by the claimed methods. Consequently, the claims so lack support (Article 6 PCT), and the application so lacks disclosure (Article 5 PCT), that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search can only be carried out for those parts of the claims for which a search is economically feasible. The search must therefore be restricted to those documents wherein reference is made to methods using promoters of defined genes having a given name (since it is not possible to find documents that relate to the use of promoters of genes defined by their sequence), i.e. the AmiC, rbcX, hliB, hsp17, nblB, rpoD, hliA, ftsH or cipB genes.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td>US 5518897 A</td>
<td>21-05-1996</td>
<td>NONE</td>
<td></td>
</tr>
<tr>
<td>JP 07079782 A</td>
<td>28-03-1995</td>
<td>NONE</td>
<td></td>
</tr>
</tbody>
</table>