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(54) Title: BACTERIAL INOCULANTS FOR ENHANCING PLANT GROWTH

(57) Abstract: A biological inoculant for enhancing the growth of plants is disclosed. The inoculant includes the bacterial strains *Herbaspirillum seropedicae* 2A, *Pantoea agglomerans* P101, *Pantoea agglomerans* P102, *Klebsiella pneumoniae* 342, *Klebsiella pneumoniae* zmvsy, *Herbaspirillum seropedicae* Z152, *Gluconacetobacter diazotrophicus* PA15, with or without a carrier. The inoculant also includes strains of the bacterium *Pantoea agglomerans* and *K. pneumoniae* which are able to enhance the growth of cereal grasses. also disclosed are the novel bacterial strains *Herbaspirillum seropedicae* 2A, *Pantoea agglomerans* P101 and P102, and *Klebsiella pneumoniae* 342 and zmvsy.

INTERNATIONAL SEARCH REPORT

Inte ıal Application No
PCT/US 01/46524

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A01N63/00 C12R1/00 C12R1/22

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 7 A01N C12R

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, WPI Data, PAJ, EPO-Internal, CHEM ABS Data, CAB Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	V.L.D.BALDANI ET AL: "Inoculation of rice plnts with the endophytic diazotrophs Herbaspirillum seropedicae and Burkholderia spp." BIOLOGY AND FERTILITY OF SOILS, vol. 30, no. 5/6, 25 February 2000 (2000-02-25), pages 485-491, XP002199370 BERLIN, DE ISSN: 0178-2762 abstract page 487 -page 491 --- -/--	1,4-7, 11,12

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in 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 document but published on or after the international filing date

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"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	Date of mailing of the international search report
22 May 2002	20. 09. 2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	LAMERS, W

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CAB [Online] CAB INTERNATIONAL, WALLINGFORD, OXON, GB; V.ARANGARASAN ET AL.: "Inoculation effects of diazotrophs and phosphobacteria on rice" retrieved from STN-INTERNATIONAL Database accession no. 1999:15719 CABA XP002199371 abstract & INDIAN JOURNAL OF MICROBIOLOGY, vol. 38, no. 2, 1998, pages 111-112, ---	1,4-7, 11,12
X	EP 1 036 492 A (AVENTIS RES & TECH GMBH & CO) 20 September 2000 (2000-09-20) page 2, line 3 - line 6 page 2, line 52 - line 55 page 3, line 1 - line 2 page 7, line 4 ---	1,4-7, 11,12
Y	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; A.K.GOEL ET AL.: "Use of biofertilizers: Potential, constraints and future strategies - a review" retrieved from EPOQUE Database accession no. PREV200000476942 XP002199372 abstract & INTERNATIONAL JOURNAL OF TROPICAL AGRICULTURE, vol. 17, no. 1-4, 1999, pages 1-18, ---	1,4-7, 11,12
Y	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; F.BASTIAN ET AL.: "Production of indole-3-acetic acid and gibberellins A1 and A3 by Acetobacter diazotrophicus and Herbaspirillum seropedicae in chemically-defined culture media" retrieved from EPOQUE Database accession no. PREV199800185888 XP002199373 abstract & PLANT GROWTH REGULATION, vol. 24, no. 1, January 1998 (1998-01), pages 7-11, --- -/--	1,4-7, 11,12

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; E.W.TRIPLETT: "Diazotrophic endophytes: Progress and prospects for nitrogen fixation in monocots" retrieved from EPOQUE Database accession no. PREV199799377209 XP002199374 abstract & PLANT AND SOIL, vol. 186, no. 1, 1996, pages 29-38, ---	1,4-7, 11,12
P,X	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; P.J.RIGGS ET AL.: "Enhanced maize productivity by inoculation with diazotrophic bacteria" retrieved from STN-INTERNATIONAL Database accession no. PREV20010502694 XP002199375 abstract & AUSTRALIAN JOURNAL OF PLANT PHYSIOLOGY, vol. 28, no. 9, 2001, pages 829-836, ---	1,4-7, 11,12
A	DATABASE CAB [Online] CAB INTERNATIONAL, WALLINGFORD, OXON, GB; E.KARPATI ET AL.: "Study of wheat and rice cultivars in association with nitrogen-fixing bacteria" retrieved from STN-INTERNATIONAL Database accession no. 2001:135540 CABA XP002199376 abstract & NOVENYTERMELES, vol. 49, no. 3, 2000, pages 233-244, ---	1,4-7, 11,12
A	DATABASE CAB [Online] CAB INTERNATIONAL, WALLINGFORD, OXON, GB; B.RETHATI ET AL.: "Characterization of Hungarian rice cultivars in nitrogen fixing associations with bacteria" retrieved from STN-INTERNATIONAL Database accession no. 2000:111719 CABA XP002199377 abstract & CEREAL RESEARCH COMMUNICATIONS, vol. 28, no. 1/2, 2000, pages 9-16, --- -/--	1,4-7, 11,12

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; R.REMUS ET AL.: "Colonization behaviour of two enterobacterial strains on cereals" retrieved from STN-INTERNATIONAL Database accession no. PREV200000182078 XP002199378 abstract & BIOLOGY AND FERTILITY OF SOILS, vol. 30, no. 5-6, March 2000 (2000-03), pages 550-557, -----	1,4-7, 11,12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/46524

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.:
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.:
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:

see additional 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, 4-7, 11, 12 (partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. Claims: 1, 4-7, 11, 12 (partially)

An inoculum for application to plants, said inoculum comprising a carrier and an effective quantity of bacteria selected from Herbaspirillum seropedicae 2A (ATCC No. PTA-2742) and Herbaspirillum seropedicae Z152 (ATCC No. 35894) and mutant strains derived therefrom, said mutant strains being able to enhance the growth of plants;
- biologically pure bacterial cultures as described in claims 4 and 5 (partially);
- a method for enhancing the growth of a plant as described in claims 6 and 7 (partially);
- a seed as described in claims 11 and 12 (partially).

2. Claims: 1, 4-7, 11-17 (partially); 3, 10 (complete)

An inoculum for application to plants other than legume plants, the inoculum comprising a carrier and an effective quantity of a Pantoea agglomerans bacterial strain.

An inoculum for application to plants, said inoculum comprising a carrier and an effective quantity of bacteria selected from Pantoea agglomerans P101 (ATCC No. PTA 2744) and Pantoea agglomerans P102 (ATCC No. PTA 2740) and mutant strains derived therefrom, said mutant strains being able to enhance the growth of plants;
- biologically pure bacterial cultures as described in claims 4 and 5 (partially);
- methods for enhancing the growth of a plant as described in claims 6, 7 (partially) and claim 10;
- a seed as described in claims 11 and 12 (partially);
- methods, an inoculum, and a seed as described in claims 13-17 (partially).

3. Claims: 1, 4-7, 11-17 (partially); 2, 8, 9 (complete)

An inoculum for application to plants, the inoculum comprising a carrier and an effective quantity of a Klebsiella pneumoniae bacterial strain.

An inoculum for application to plants, said inoculum comprising a carrier and an effective quantity of bacteria selected from Klebsiella pneumoniae 342 (ATCC No. PTA-2743) and Klebsiella pneumoniae zmvsy (ATCC No. PTA-2741) and mutant strains derived therefrom, said mutant strains being able to enhance the growth of plants;
- biologically pure bacterial cultures as described in claims 4 and 5 (partially);
- methods for enhancing the growth of a plant as described in claims 6,7 (partially) and claims 8 and 9;

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

- a seed as described in claims 11 and 12 (partially);
- methods, an inoculum, and a seed as described in claims 13-17 (partially).

4. Claims: 1, 6, 7, 11, 12 (partially)

An inoculum for application to plants, said inoculum comprising a carrier and an effective quantity of bacteria selected from Gluconacetobacter diazotrophicus PA15 (ATCC No. 49037) and mutant strains derived therefrom, said mutant strains being able to enhance the growth of plants;
- a method for enhancing the growth of a plant as described in claims 6 and 7 (partially);
- a seed as described in claims 11 and 12 (partially).

INTERNATIONAL SEARCH REPORT

Information on patent family members

rnational Application No
CT/US 01/46524

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1036492 A	20-09-2000	AU 3555000 A	04-10-2000
		BG 105879 A	28-06-2002
		BR 0008982 A	26-12-2001
		CZ 20013302 A	13-03-2002
		WO 0054568 A	21-09-2000
		EP 1164826 A	02-01-2002