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(54) Title: MICROBIALS FOR BACTERIAL INHIBITION

<i>Escherichia coli</i>	CM256	CM857	CM978	CM1004	MDG1607
Field Isolate 1	+	+	+	+	+
Field Isolate 2	+	+/-	+	+/-	+
Field Isolate 3	+	+/-	+	+/-	+
Field Isolate 4	+	+/-	+	+	+
Field Isolate 5	+	+/-	+	+	+
Field Isolate 6	+/-	+/-	+	+	+
Field Isolate 7	+	+/-	+/-	+/-	+
Field Isolate 8	+	+/-	+	+	+
Field Isolate 9	+	+	+	+/-	+
Field Isolate 10	+	+	+	+	+
Field Isolate 11	+	+	+	+	+
Field Isolate 12	+/-	+	+	+	+
Field Isolate 13	+/-	+	+	+	+
Field Isolate 14	+	+/-	+/-	+/-	+
Field Isolate 15	+	+/-	+/-	+	+
Field Isolate 16	+	+/-	+	+	+
Field Isolate 17	+	+/-	+	+	+
Field Isolate 18	+	+/-	+	+	+
Field Isolate 19	+	+/-	+	+/-	+
Field Isolate 20	+/-	+/-	+	+/-	+
Field Isolate 21	+/-	+/-	+	+/-	+
Field Isolate 22	+/-	+/-	+	+	+
Field Isolate 23	+/-	+/-	+	+	+
Field Isolate 24	+	+	+/-	+/-	+
Field Isolate 25	+	+/-	+	+/-	+
Field Isolate 26	+	+/-	+	+/-	+
Field Isolate 27	+	+/-	+	+/-	+
Field Isolate 28	+	+/-	+	+/-	+
Field Isolate 29	+	+/-	+	+/-	+
Field Isolate 30	+	+	+	+/-	+
Field Isolate 31	+	+/-	+	+	+
Field Isolate 32	+	+/-	+/-	+	+

Fig. 1A

(57) Abstract: The invention relates to direct-fed microbials for use in *E. coli*, *Salmonella*, and/or *Clostridium* inhibition in animals. More particularly, the invention relates to isolated *Bacillus* strains CM256 (NRRL No. B-67706), CM857 (NRRL No. B-67705), CM978 (NRRL No. B-67703), CM1004 (NRRL No. B-67708), and MDG1607 (NRRL No. B-67666), and strains having all of the identifying characteristics of these strains, for a use comprising the above-mentioned use. The invention also relates to the use of isolated *Bacillus* strains CM256 (NRRL No. B-67706), CM857 (NRRL No. B-67705), CM978 (NRRL No. B-67703), and CM1004 (NRRL No. B-67708), and strains having all of the identifying characteristics of these strains, to treat plants having diseases caused by *E. coli*, *Salmonella*, and/or *Clostridium*.



WO 2024/254478 A3

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

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

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08 May 2025 (08.05.2025)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 24/33048

A. CLASSIFICATION OF SUBJECT MATTER
IPC - INV. A61K 35/742, A23K 10/18 (2024.01)
ADD. A23K 50/10 (2024.01)

CPC - INV. A23K 10/18, A61K 35/742

ADD. A23K 50/10, A23L 33/135, A61P 31/04

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.
A	US 2021/0236563 A1 (MICROBIAL DISCOVERY GROUP, LLC.) 5 August 2021 (05.08.2021) abstract, para [0003], [0006], [0128]	1-7, 28, 83-88, 109
A	US 2010/0093860 A1 (UNIVERSITEIT GENT) 15 April 2010 (15.04.2010) abstract	1-7, 28, 83-88, 109
A	US 2020/0276279 A1 (DUPONT NUTRITION BIOSCIENCES APS) 3 September 2020 (03.09.2020) abstract	1-7, 28, 83-88, 109

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"D" document cited by the applicant in the international application	"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
"E" earlier application or patent but published on or after the international filing date	"&" document member of the same patent family
"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	

Date of the actual completion of the international search
6 October 2024

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 24/33048

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.: 9-22, 27, 36-49, 54, 61, 64-65, 67-82, 90-103 and 108
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:

----- See 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.: 1-7, 28, 83-88 and 109, limited to *Bacillus* strain CM256 (NRRL No. B-67706)

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 24/33048

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+: 1-8, 23-26, 28, 83-89, 104-107, and 109, directed to a method of feeding an animal comprising administering to the animal a feed composition or drinking water comprising an effective amount of an additive comprising a Bacillus strain, wherein said strain causes E. coli, Salmonella, and/or Clostridium inhibition in the animal. The animal feeding method will be searched to the extent that the animal is a poultry species and the Bacillus strain is CM256 (NRRL No. B-67706). The first named invention was determined based on alternative NRRL Bacillus strains in claim 1. This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. It is believed that claims 1-7, 28, 83-88 and 109, limited to said animal feeding method encompass this first named invention, and thus these claims will be searched without fee to the extent that animal feeding method encompasses Bacillus strain CM256 (NRRL No. B-67706). Additional animal feeding methods will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected animal feeding methods. 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 animal feeding method wherein the animal is a porcine species and the Bacillus strain is CM857 (NRRL No. B-67705) (claims 1-3, 5, 6-7 (in part), 8, 25-26, 28, 83-85, 87-88 (in part), 89, 106, 107 and 109).

Group II: claims 29-35, 50-53, 55, directed to a method of controlling a detrimental effect of E. coli, Salmonella, and/or Clostridium, the method comprising the steps of administering to an animal a feed composition or drinking water comprising an effective amount of an additive comprising an isolated Bacillus strain, thereby controlling said detrimental effect.

Group III+: claims 56, 60 (in part), and 66, directed to a composition comprising a commercial package comprising an isolated Bacillus strain used as an additive for feed or drinking water of an animal. Group II+ will be searched upon payment of additional fees. The Bacillus feed additive composition may be searched, for example, to the extent that the Bacillus strain is CM256 (NRRL No. B-67706). It is believed that claims 56, 60 (in part) and 66 limited to said Bacillus strain, read on this exemplary invention. Additional animal feed additive compositions will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected animal feed additive compositions. 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 animal feed additive composition comprising the Bacillus strain CM857 (NRRL No. B-67705) (Claims 56, 60 (in part) and 66).

Group IV: Claims 57-59, 60 (in part), and 62-63, directed to a feed additive composition for an animal feed or water comprising an isolated Bacillus strain.

Group V+: Claims 110-118, directed to a method of treating a plant to inhibit a bacterial disease caused by E. coli, Salmonella, and/or Clostridium, comprising contacting the plant with a composition comprising an effective amount of an isolated Bacillus strain, thus inhibiting E. coli, Salmonella, and/or Clostridium in the plant. Group V+ will be searched upon payment of additional fees. The plant treatment method may be searched, for example, to the extent that the Bacillus strain encompasses CM256 (NRRL No. B-67706). It is believed that claims 110-112 limited to said plant treatment method, read on this exemplary invention. Additional plant treatment methods will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected plant treatment methods. 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 a plant treatment method wherein the Bacillus strain encompasses CM857 (NRRL No. B-67705) (Claims 110-111, and 113).

The inventions listed as Groups I+, II, III+, IV and V+ 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:

No technical features are shared between the NRRL Bacillus strains of Groups I+, III+ and V+, accordingly, these groups lack unity a priori.

Additionally, even if the inventions listed as Group I+ were considered to share technical features, these shared technical features are previously disclosed by the prior art, as further discussed below.

Special Technical Features

Group I+ requires a method of feeding an animal comprising administering to the animal a feed composition or drinking water comprising an effective amount of an additive comprising a Bacillus strain, not required by groups II, III+, IV and V+.

Group II requires a method of controlling a detrimental effect of E. coli, Salmonella, and/or Clostridium, not required by groups I+, III+, IV and V+.

Group III+ requires a composition comprising a commercial package comprising an isolated Bacillus strain, not required by groups I+, II and IV and V+.

Group IV requires a feed additive composition for an animal feed or water, not required by groups I+, II, III+ and V+.

Group V+ requires a method of treating a plant to inhibit a bacterial disease caused by E. coli, Salmonella, and/or Clostridium, not required by groups I+, II, III+ and IV.

***** See Next Extra Sheet to continue *****

INTERNATIONAL SEARCH REPORT

International application No.

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continuation of previous extra sheet:

Common Technical Features

The common technical feature shared by Groups I+, II, III+, IV and V+, is Bacillus strains (and compositions thereof) capable of inhibiting E. coli, Salmonella, and/or Clostridium. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by US 2021/0236563 A1 to Microbial Discovery Group LLC (hereinafter "MDG"). MDG teaches said Bacillus strains (abstract "microbials for use in E. coli, Salmonella, and/or Campylobacter inhibition ... the invention relates to isolated Bacillus strains", para [0006] "compositions are provided for inhibiting E. coli, Salmonella, and Campylobacter").

The common technical feature shared by Groups I+ and II is a method for inhibiting E. coli, Salmonella, and/or Clostridium by administering to an animal a feed composition comprising a Bacillus strain. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by MDG (para [0003] "The present invention relates to direct-fed microbial (DFM) compositions and methods for E. coli, Salmonella, and Campylobacter inhibition in an animal.", para [0128] "A method of feeding an animal, the method comprising the step of administering to the animal a feed composition or drinking water comprising an effective amount of an additive comprising an isolated Bacillus strain").

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.

Groups I+, II, III+, IV and V+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

RE: Item 4: claims 9-22, 27, 36-49, 54, 61, 64-65, 67-82, 90-103 and 108 are not drafted in accordance with the second and third sentences of Rule 6.4(a) regarding multiply dependent claims.

Note: Claim 118 is objected to for lack of antecedent basis. As drafted, claim 118 depends from claims 109 or 110, however, claim 109 fails to recite treating a plant. For the purposes of this invitation, claim 118 is construed as though depending from claims 110-111 (as in claims 112-117).