Data Article

Data associated with the characterization and presumptive identification of *Bacillus* and related species isolated from honey samples by using HiCrome *Bacillus* agar

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A R T I C L E   I N F O

A B S T R A C T

The dataset described in this paper provides information on the morphological features of 24 different species of the genera *Bacillus, Paenibacillus, Brevibacillus, Lysinibacillus,* and *Rummeliibacillus* when growing in HiCrome Bacillus agar. The species studied are common contaminants of honey. In support to the recent publication entitled "HiCrome Bacillus agar for presumptive identification of Bacillus and related species isolated from honey samples" (2), a collection of 197 bacterial isolates belonging to 24 different species of aerobic spore-forming bacteria have been screened for their colony appearance and color and any substrate color change of HiCrome Bacillus agar at 24 and 48 h of incubation. Two simple flowcharts utilizing a combination of colony and media characteristics in the chromogenic medium and a set of simple biochemical and morphological tests were developed for quick presumptive identification.

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Specifications table

Subject area	Microbiology, Food Microbiology
More specific subject area	Microbiological Methods, Bioinformatics
Type of data	Tables, Figures, Flowcharts
How data was acquired	Digital camera, PCR, purification, sequencing, and Phylogenetic analysis
Data format	Analyzed
Experimental factors	Isolation of spore-forming bacteria
	Genomic DNA from pure bacterial cultures
Experimental features	Isolation and cultivation of bacteria, 16S rRNA sequencing, microbiological tests, colony morphology, and microscopy
Data source location	Bacteria were isolated from samples from different geographical areas
	The analysis was performed at CIDEFI - Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Calles 60 y 119 S/N, 1900 La Plata, Buenos Aires, Argentina
Data accessibility	Data are available with this article.
	16S rRNA sequences of selected bacterial strains (n = 56) isolated from honey or honeybee larvae have been deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under accession numbers summarized in Table 1.
Related research article	HiCrome Bacillus agar for presumptive identification of *Bacillus* and related species isolated from honey samples by Alippi and Abrahamovich (*International Journal of Food Microbiology, 2019, DOI: 10.1016/j.ijfoodmicro.2019.108245*)

Value of the data
- Data presented to describe the colony appearance on HiCrome Bacillus agar of several *Bacillus* and related species commonly found in honey.
- Data generated serve as a point of reference for further research in microbial diversity, microbial ecology, and microbial taxonomy.
- The information presented could be beneficial for other researchers that are interested in the microbiology of honey and is potentially also of benefit for research on other food products.

1. Data

The dataset described in this paper provides information on the morphological features of 24 different species of the genera *Bacillus, Paenibacillus, Brevibacillus, Lysinibacillus,* and *Rummeliibacillus* when growing in HiCrome Bacillus agar. The species studied here have been previously reported in honey [1—3,5,7,10,12—15].

A collection of 197 bacterial isolates belonging to the 24 species tested have been screened for their colony appearance and color and any substrate color change of HiCrome Bacillus agar at 24 and 48 h of incubation (Fig. 1 and Table 3). Colors of colonies and substrate observed were compared with a Pantone international chart and identified with a PMS number (http://www.cal-print.com/InkColorChart.htm).

The Ecometric technique was used for comparative evaluation of HiCrome Bacillus agar and the control medium (Figs. 2 and 3 and Table 3). E-values (Table 3) were obtained for 28 selected isolates tested by using previously published methods [2,8].

Two Flowcharts were prepared by a combination of colony and media characteristics in HiCrome Bacillus agar and a set of selected biochemical and morphological tests that are used routinely in Microbiological laboratories. The first chart (Fig. 4) permits the identification of the aerobic spore-forming bacteria reported in honey by a few simple tests. The more simplified flowchart presented in Fig. 5 allows differentiating typical strains of aerobic spore-forming species by direct isolation from honey.

The bacterial identity of selected strains isolated from honey or honeybee larvae (n = 56) (Table 1) were confirmed by sequencing the 16S rDNA. Sequences were deposited in the DDBJ/EMBL/Genbank under the Accession Numbers listed in Table 1. For comparisons, 16S rRNA sequences from type cultures (n = 32 plus 1 outlier) were used and are listed in Table 2.
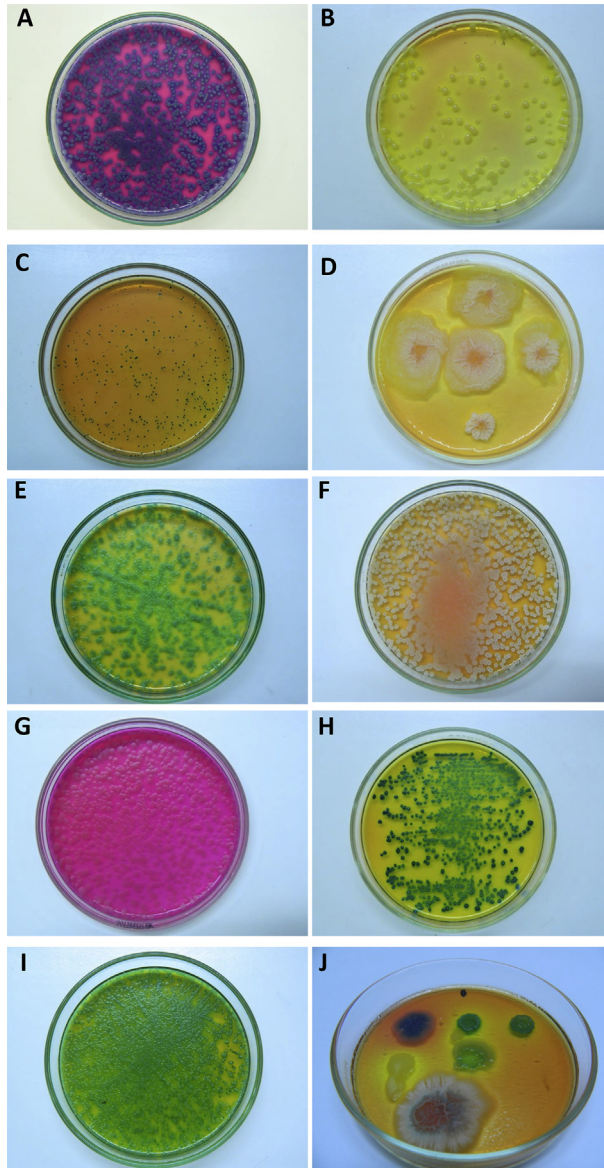
Fig. 1. Comparison of colony appearance on HiCrome Bacillus agar of several *Bacillus* and related species commonly found in honey: A. *Bacillus cereus* m87, B. *Bacillus megaterium* m344, C. *Paenibacillus alvei* mv82, D. *Bacillus amyloliquefaciens* m39, E. *Bacillus subtilis* ATCC 7061, F. *Bacillus subtilis* m191, G. *Lysinibacillus sphaericus* m533, H. *Bacillus licheniformis* mv68, I. *Bacillus pumilus* mv49b, J. Complex sample of naturally contaminated honey containing (Clockwise from upper left) *B. cereus, B. licheniformis, B. pumilus B. amyloliquefaciens,* and *B. megaterium.*

2. Experimental design, materials, and methods

A collection of 197 bacterial isolates of *Bacillus, Brevibacillus, Lysinibacillus, Paenibacillus,* and *Rummeliibacillus* belonging to different species that have been reported in honey [1−3,5,7,10,12−15] were screened for their abilities to grow and colony appearance and color, and any substrate color change by using HiCrome Bacillus agar. The collection includes 167 isolates from honey samples from
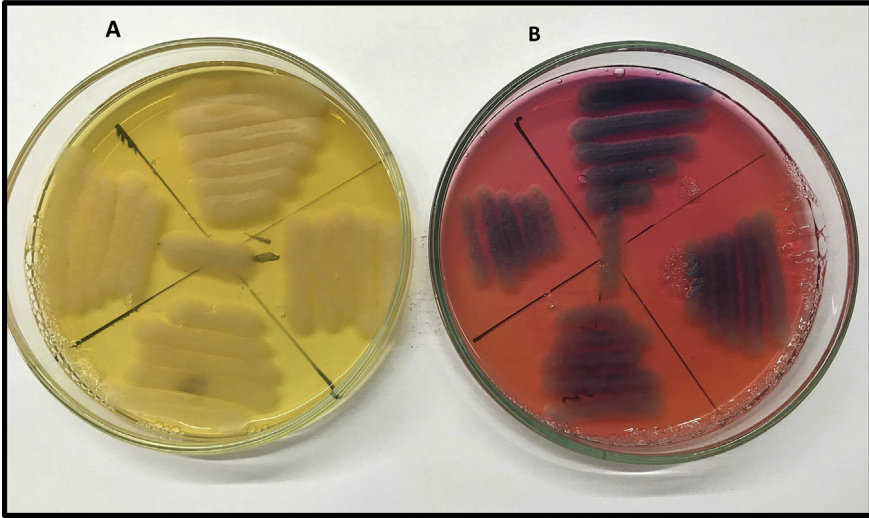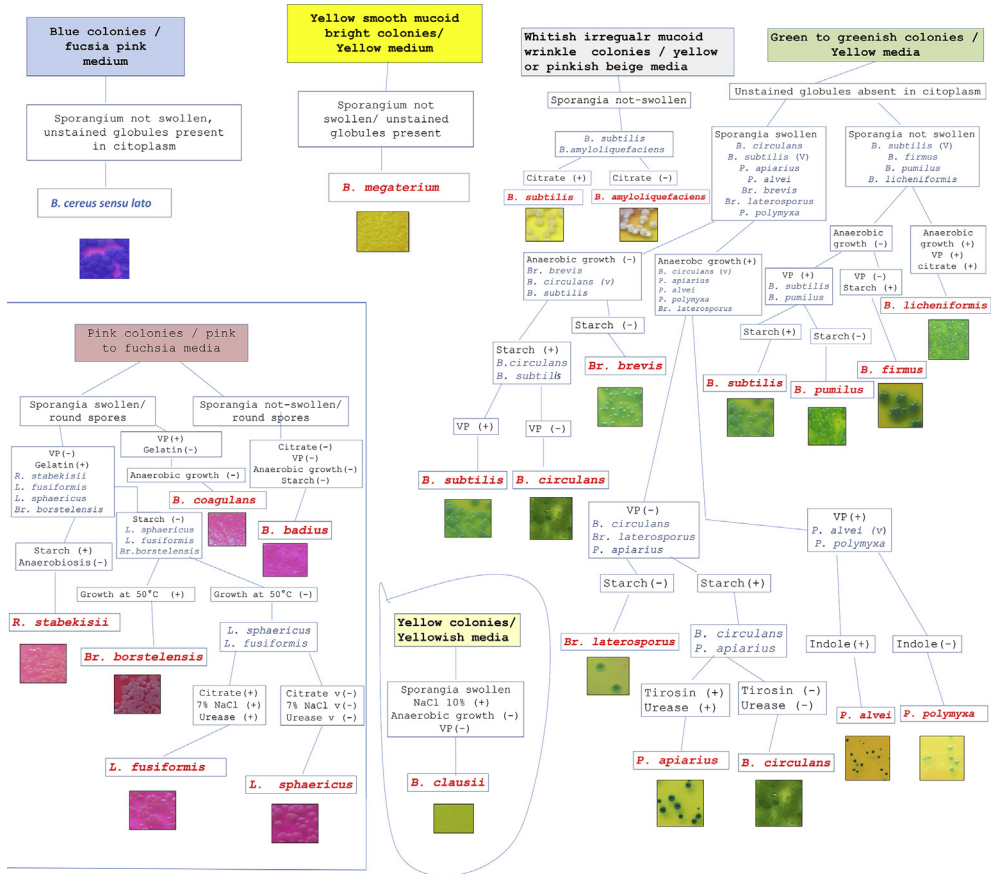
Fig. 2. *Bacillus cereus* ATCC 11778 growing on A: BHIT and B. HiCrome Bacillus agar showing luxuriant growth (+++) and E value = 5.
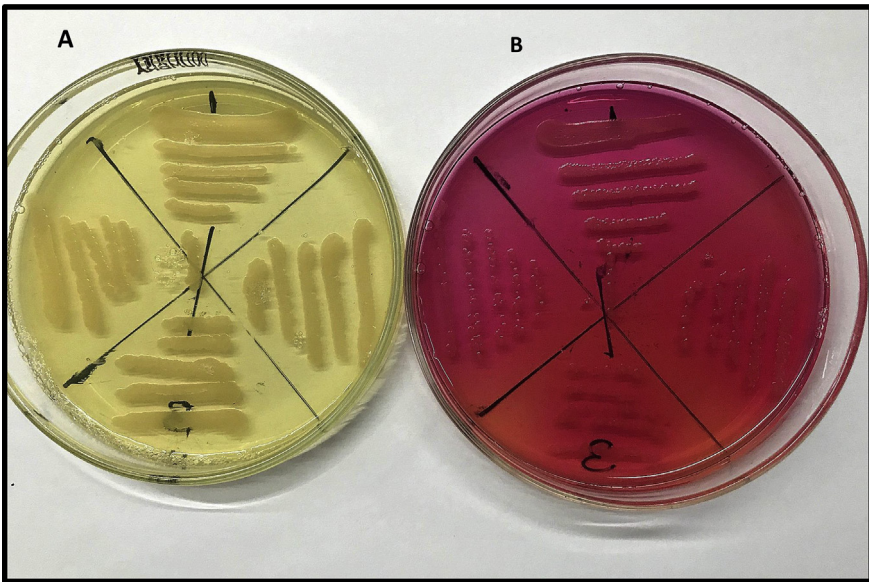
Fig. 3. *Rummeliibacillus stabekisii* mv111 growing on A: MYPGP and B. HiCrome Bacillus agar showing good growth (++) and E value = 5.

different geographical areas including Argentina, Brazil, France, Italy, and Mexico; 9 isolates from honeybee larvae from different geographical areas including Argentina, France and Sweden; and 21 strains from Culture Collections used for comparison and quality control. Bacteria were maintained as stock cultures at −80 °C in the correspondent broth medium, Müller-Hinton Broth, yeast extract, potassium phosphate, glucose, and pyruvate (MYPGP) [4] or Brain heart infusion (BHI) plus 20% glycerol (v/v). For short-term storage, the strains were kept at 4 °C in screw-capped vials containing MYPGP or BHI semi-solid (0.4% agar).

Fig. 4. Flowchart - Main steps for the identification of common strains of *Bacillus* and related species from honey by using a combination of selected morphological and biochemical tests and HiCrome Bacillus agar.

Bacterial smears stained by Schaeffer-Fulton technique were examined for the presence and location of spores within cells, as well as for the size and shape of vegetative cells [9,11]. Also, the presence of unstained globules in the cytoplasm [6,9,11] was examined by phase contrast microscopy (Leica, ICC50).

Bacterial cultures were also tested by catalase reaction, anaerobic growth, nitrate reduction, Voges-Proskauer reaction (VP), pH in VP broth, indol and urease production, mannitol, L-arabinose, and citrate utilization, starch and gelatin hydrolysis, decomposition of tyrosine, growth in 7% and 10% of NaCl and at different temperatures (30-37-50 and 55 °C) according to standard protocols [6,9,11].

The Ecometric technique was used for comparative evaluation of HiCrome Bacillus agar and the correspondent control media (BHI or MYPGP). Overnight cultures were adjusted to 0.5 Mc Farland in sterile distilled water. One loop of 10 μl of each suspension was sequentially diluted from streak to

Fig. 5. Flowchart - Simplified steps for the identification of common strains of *Bacillus* and related species from honey by using isolation in HiCrome Bacillus agar and selected morphological and biochemical tests.

streak onto each medium by inoculating 21 streaks (5 per quadrant and 1 in the center). Growth on the plates was recorded as a score. Readings were presented as absolute growth indices with possible values of 0−5, where 0 is an absence of growth in any streak and 5 was the maximum score obtained when all of the streaks in the four quadrants and also the last streak showed visible bacterial growth [2,8]. Twenty-eight bacterial strains with different colony types (Table 2) were used for the evaluation. Plates were inoculated and incubated in duplicate for 24−48 h at 37 °C. Scores for HiCrome and control plates were compared to estimate the degree of inhibition due to the chromogenic mixture (Table 3, Figs. 2 and 3).

The identity of selected strains (n = 56) was confirmed by sequencing the 16S rDNA. Universal eubacterial primers used for 16S rDNA sequence analysis were 27f (5′-AGAGTTTGATCMTGGCTCAG - 3′) and 1492r (5′-TACGGYTACCTTGTTACGACTT- 3′).

For purification of PCR products the following enzymatic procedure was used: The mixture contained 0.5 μl Antarctic phosphatase buffer (NEB, Migliore Lacaustra, Argentina), 0.6 μl Antarctic phosphatase (NEB, Migliore Lacaustra, Argentina), 0.6 μl Exonuclease I (NEB, Migliore Lacaustra, Argentina), 4 μl unpurified PCR product and 3.3 μl double distilled sterile deionized water. The Thermal cycler protocol consisted of one step of 37 °C for 20 min and the second of 80 °C for 20 min.

The quality and quantity of PCR products were assessed by gel electrophoresis (1 μl/1.6% agarose/ molecular weight marker QuantiMarker, Promega, Argentina) and DNA concentration was estimated

Table 1
Source and accession numbers of bacterial strains and isolates used in this study.

Species	Strain/Isolate designation	Source and geographical origin	Accesion number
Bacillus amyloliquefaciens	xx	Honeybee larvae-Argentina	KP177517.1
Bacillus amyloliquefaciens	mv35	Honey- Argentina	MG004186.1
Bacillus amyloliquefaciens	m39	Honey- Argentina	MG004187.1
Bacillus amyloliquefaciens	m163b	Honey- Argentina	MG004188.1
Bacillus amyloliquefaciens	m164b	Honey- Argentina	MG004193.1
Bacillus amyloliquefaciens	m287b	Honey- Argentina	MG004189.1
Bacillus amyloliquefaciens	m291b	Honey- Argentina	MG004190.1
Bacillus badius	CCT 0196	CCT	N/A
Bacillus cereus	ATCC 11778	ATCC	AF290546.1
Bacillus cereus	cm4	Honey- Argentina	N/A
Bacillus cereus	m6c	Honey- Argentina	KP005456.1
Bacillus cereus	cm7	Honey- Argentina	N/A
Bacillus cereus	cm8	Honey- Argentina	N/A
Bacillus cereus	m9a	Honey- Argentina	N/A
Bacillus cereus	m10a	Honey- Argentina	N/A
Bacillus cereus	m10b	Honey- Argentina	N/A
Bacillus cereus	m12	Honey- Argentina	N/A
Bacillus cereus	m19	Honey- Argentina	N/A
Bacillus cereus	m21	Honey- Argentina	N/A
Bacillus cereus	m28	Honey - Argentina	N/A
Bacillus cereus	m31	Honey- Argentina	N/A
Bacillus cereus	mv33	Honey- Argentina	KU230015.1
Bacillus cereus	cm37	Honey- Argentina	N/A
Bacillus cereus	mv39b	Honey- Argentina	N/A
Bacillus cereus	mv41x	Honey- Argentina	N/A
Bacillus cereus	mv54	Honey- Argentina	N/A
Bacillus cereus	m54	Honey- Argentina	N/A
Bacillus cereus	mv67	Honey- Argentina	N/A
Bacillus cereus	m73	Honey- Argentina	N/A
Bacillus cereus	mv73	Honey- Argentina	N/A
Bacillus cereus	mv75	Honey- Argentina	N/A
Bacillus cereus	mv76	Honey- Argentina	N/A
Bacillus cereus	mv77	Honey- Argentina	N/A
Bacillus cereus	mv78	Honey- Argentina	N/A
Bacillus cereus	mv79	Honey- Argentina	N/A
Bacillus cereus	mv80	Honey- Argentina	N/A
Bacillus cereus	m84	Honey- Argentina	N/A
Bacillus cereus	m85	Honey- Argentina	N/A
Bacillus cereus	mv86	Honey- Argentina	N/A
Bacillus cereus	mv87	Honey- Argentina	N/A
Bacillus cereus	m90	Honey- Argentina	N/A
Bacillus cereus	m91	Honey- Argentina	N/A
Bacillus cereus	m97	Honey- Argentina	N/A
Bacillus cereus	m105	Honey- Argentina	N/A
Bacillus cereus	mv114	Honey- Argentina	N/A
Bacillus cereus	mv117	Honey- Argentina	N/A
Bacillus cereus	cm117	Honey- Argentina	N/A
Bacillus cereus	cm118	Honey- Argentina	N/A
Bacillus cereus	m134	Honey- Argentina	N/A
Bacillus cereus	m139b	Honey- Argentina	N/A
Bacillus cereus	m143b	Honey- Argentina	N/A
Bacillus cereus	m143c	Honey- Argentina	N/A
Bacillus cereus	m157	Honey- Italy	N/A
Bacillus cereus	m158	Honey- Argentina	N/A
Bacillus cereus	m163a	Honey- Argentina	N/A
Bacillus cereus	m167	Honey- Argentina	N/A
Bacillus cereus	m189	Honey- Argentina	N/A
Bacillus cereus	m193	Honey- Argentina	N/A
Bacillus cereus	m225a	Honey- Argentina	N/A
Bacillus cereus	m228	Honey- Argentina	N/A

Table 1 (*continued*)

Species	Strain/Isolate designation	Source and geographical origin	Accesion number
Bacillus cereus	m243	Honey- Argentina	N/A
Bacillus cereus	m244	Honey- Argentina	N/A
Bacillus cereus	m248	Honey- Argentina	N/A
Bacillus cereus	m262	Honey- Argentina	N/A
Bacillus cereus	m267	Honey- Argentina	N/A
Bacillus cereus	cm281	Honey- Argentina	N/A
Bacillus cereus	m282a	Honey- Argentina	N/A
Bacillus cereus	m287a	Honey- Argentina	N/A
Bacillus cereus	m292	Honey- Argentina	N/A
Bacillus cereus	m296	Honey- Argentina	N/A
Bacillus cereus	m298	Honey- Argentina	N/A
Bacillus cereus	m305	Honey- Argentina	N/A
Bacillus cereus	m308	Honey- Argentina	N/A
Bacillus cereus	m309	Honey- Argentina	N/A
Bacillus cereus	m316	Honey- Argentina	N/A
Bacillus cereus	m365	Honey- Argentina	N/A
Bacillus cereus	m370	Honey- Argentina	N/A
Bacillus cereus	m383	Honey- Argentina	N/A
Bacillus cereus	m385	Honey- Argentina	N/A
Bacillus cereus	m387	Honey- Argentina	KP005455.1
Bacillus cereus	m388	Honey- Argentina	N/A
Bacillus cereus	m434	Honey- Argentina	KU230027.1
Bacillus cereus	m436	Honey- Argentina	N/A
Bacillus cereus	m437b	Honey- Argentina	N/A
Bacillus cereus	m438	Honey- Argentina	N/A
Bacillus cereus	m439	Honey- Argentina	N/A
Bacillus cereus	m444	Honey- Argentina	N/A
Bacillus cereus	m445b	Honey - Argentina	N/A
Bacillus cereus	LPcer1	Honeybee larvae- Argentina	KX431225.1
Bacillus cereus	MexB	Honey- Mexico	KU230012.1
Bacillus cereus	MexC	Honey- Mexico	KU230013.1
Bacillus circulans	ATCC 4515	ATCC	N/A
Bacillus clausii	Fr231	Honey- France	KU230014.1
Bacillus clausii	m448b	Honey- Brazil	KX685159.1
Bacillus coagulans	NRRL NRS 609	NRRL	N/A
Bacillus firmus	ATCC 8247	ATCC	N/A
Bacillus licheniformis	mv55	Honey-Argentina	KU230018.1
Bacillus licheniformis	mv68	Honey-Argentina	MF187633.1
Bacillus licheniformis	mv72	Honey- Argentina	N/A
Bacillus licheniformis	m112	Honey- Argentina	N/A
Bacillus licheniformis	NRRL B-1001	NRRL	N/A
Bacillus megaterium	m280	Honey- Argentina	N/A
Bacillus megaterium	m327	Honey- Argentina	MF187637.1
Bacillus megaterium	m344	Honey- Argentina	N/A
Bacillus megaterium	m373	Honey- Argentina	N/A
Bacillus megaterium	m435	Honey- Mexico	KU230028.1
Bacillus megaterium	m441	Honey- Argentina	N/A
Bacillus megaterium	m458	Honey- Brazil	N/A
Bacillus megaterium	NRRL B-939	NRRL	N/A
Bacillus mycoides	m336	Honey- Argentina	MF187638.1
Bacillus mycoides	m425	Honey- Argentina	N/A
Bacillus mycoides	ATCC 10206	ATCC	N/A
Bacillus pumilus	mv41aA	Honey- Argentina	MG366818.1
Bacillus pumilus	mv49b	Honey- Argentina	KU230016.1
Bacillus pumilus	mv74	Honey- Argentina	MF972935.1
Bacillus pumilus	mv81	Honey- Argentina	KU230019.1
Bacillus pumilus	m108	Honey- Argentina	N/A
Bacillus pumilus	m116	Honey- Argentina	KU230020.1
Bacillus pumilus	m157	Honey- Italy	N/A
Bacillus pumilus	m187	Honey- Argentina	N/A
Bacillus pumilus	m225b	Honey- Argentina	N/A
Bacillus pumilus	m288	Honey- Argentina	MF187635.1

Table 1 (*continued*)

Species	Strain/Isolate designation	Source and geographical origin	Accesion number
Bacillus pumilus	m330	Honey- Argentina	MF187646.1
Bacillus pumilus	m335	Honey- Argentina	N/A
Bacillus pumilus	m339	Honey- Argentina	MG366884.1
Bacillus pumilus	m350	Honey- Argentina	KU230023.1
Bacillus pumilus	m354	Honey- Argentina	N/A
Bacillus pumilus	m357	Honey- Argentina	MF187634.1
Bacillus pumilus	m358	Honey- Argentina	MG345110.1
Bacillus pumilus	m360	Honey- Argentina	MF187636.1
Bacillus pumilus	m363	Honey- Argentina	KU230024.1
Bacillus pumilus	m414	Honey- Argentina	KU230026.1
Bacillus pumilus	ATCC 7061[T]	ATCC	AY876289.1
Bacillus subtilis	m11	Honey- Argentina	N/A
Bacillus subtilis	m13	Honey - Argentina	MF187645.1
Bacillus subtilis	m45	Honey- Argentina	N/A
Bacillus subtilis	cm45	Honey- Argentina	MF187639.1
Bacillus subtilis	mv49a	Honey- Argentina	N/A
Bacillus subtilis	mv51	Honey- Argentina	N/A
Bacillus subtilis	mv53b	Honey- Argentina	N/A
Bacillus subtilis	mv63	Honey- Argentina	N/A
Bacillus subtilis	mv64	Honey- Argentina	N/A
Bacillus subtilis	mv65	Honey- Argentina	N/A
Bacillus subtilis	mv66	Honey- Argentina	N/A
Bacillus subtilis	mv70	Honey- Argentina	N/A
Bacillus subtilis	mv71	Honey- Argentina	N/A
Bacillus subtilis	m107	Honey- Argentina	N/A
Bacillus subtilis	m117	Honey- Argentina	N/A
Bacillus subtilis	m119	Honey- Argentina	N/A
Bacillus subtilis	m191	Honey- Argentina	MF187644.1
Bacillus subtilis	m192	Honey- Argentina	N/A
Bacillus subtilis	m197	Honey- Argentina	N/A
Bacillus subtilis	m291b	Honey- Argentina	N/A
Bacillus subtilis	m329	Honey- Argentina	KU230021.1
Bacillus subtilis	m334	Honey- Argentina	KU230022.1
Bacillus subtilis	m347	Honey- Argentina	KP177515.1
Bacillus subtilis	m351	Honey- Argentina	KP177516.1
Bacillus subtilis	m384	Honey- Argentina	N/A
Bacillus subtilis	m386	Honey- Argentina	N/A
Bacillus subtilis	m389	Honey- Argentina	N/A
Bacillus subtilis	m392	Honey- Argentina	MF187640.1
Bacillus subtilis	m412	Honey- Argentina	N/A
Bacillus subtilis	NRRL B-543	NRRL	N/A
Bacillus thuringiensis	ATCC 10792[T]	ATCC	D16281.1
Bacillus thuringiensis	m5	Honey - Argentina	N/A
Bacillus thuringiensis	mv50b	Honey - Argentina	KU230017.1
Bacillus thuringiensis	m391	Honey - Argentina	N/A
Bacillus thuringiensis	m395	Honey- Argentina	KU230025.1
Bacillus thuringiensis	m401	Honey- Argentina	N/A
Brevibacillus borstelensis	m348	Honey- Argentina	MF187641.1
Brevibacillus borstelensis	RC	Honey- Argentina	KP177514.1
Brevibacillus brevis	ATCC 8246	ATCC	N/A
Brevibacillus laterosporus	CCT 0031	CCT	N/A
Brevibacillus laterosporus	BLAT169	Honeybee larvae - Argentina	KX102627.1
Brevibacillus laterosporus	BLAT170	Honeybee larvae - Argentina	KX431223.1
Brevibacillus laterosporus	BLAT171	Honeybee larvae - Argentina	KX431224.1
Lysinibacillus fusiformis	mv119	Honey- Argentina	MG004185.1
Lysinibacillus sphaericus	ATCC 245	ATCC	N/A
Lysinibacillus sphaericus	m533	Honey- Argentina	MG001492.1
Lysinibacillus sphaericus	LMDZA	Honeybee larvae - Argentina	MG004191.1
Paenibacillus alvei	NRRL B-383	NRRL	N/A
Paenibacillus alvei	mv82	Honey- Argentina	MF187643.1
Paenibacillus alvei	m291a	Honey- Argentina	MF187632.1

Table 1 (*continued*)

Species	Strain/Isolate designation	Source and geographical origin	Accesion number
Paenibacillus alvei	m420	Honey- Argentina	MF187642.1
Paenibacillus amylolyticus	NRRL B-14940	NRRL	N/A
Paenibacillus apiarius	ATCC 29575	ATCC	N/A
Paenibacillus larvae ERIC I	ATCC 9545^{T}	ATCC	NR_118956.1
Paenibacillus larvae ERIC IV	ATCC 13537^{T}	ATCC	KT363749.1
Paenibacillus larvae ERIC I	PL38	Honeybee larvae-Argentina	N/A
Paenibacillus larvae ERIC I	PL45	Honeybee larvae- France	N/A
Paenibacillus larvae ERIC I	PL58	Honeybee larvae- Sweden	N/A
Paenibacillus larvae ERIC II	SAG 290	Honey - Unknown	N/A
Paenibacillus larvae ERIC II	SAG 10367	Honey- Unknown	CP020557
Paenibacillus larvae ERIC II	SAG 10754	Honey- Unknown	N/A
Paenibacillus polymyxa	NRRL B-510	NRRL	N/A
Rummeliibacillus stabekisii	mv111	Honey- Argentina	MF972934.1

ATCC: American Type Culture Collection, USA; CCT: Colleção de Culturas Tropical, Brazil; NRRL: Northern Utilization Research and Development Division, USA; SAG: Servicio Agrícola Ganadero, Chile.
N/A: Not applicable.

Table 2
Accession numbers of 16 S rRNA sequences from Type cultures used for sequence analysis.

| Species	Strain	Accession number
Bacillus amyloliquefaciens	NBRC 15535	NR_112685.1
Bacillus badius	ATCC 14574	X77790.1
Bacillus cereus	ATCC 11778	NR_074540.1
Bacillus circulans	ATCC 4513	AY724690.1
Bacillus clausii	DSM 8716	X76440.1
Bacillus coagulans	ATCC 7050	DQ297928.1
Bacillus firmus	NBRC 15306	NR_112635.1
Bacillus flexus	IFO15715	NR_024691.1
Bacillus licheniformis	ATCC 14580	NR_074923.1
Bacillus megaterium	IAM 13418	D16273.1
Bacillus mycoides	ATCC 6462	NR_115993.1
Bacillus niabensis	4T19	AY998119.2
Bacillus pumilus	ATCC 7061	AY876289.1
Bacillus simplex	DSM 1321	AJ439078
Bacillus subtilis	DSM 10	JQ424889.1
Bacillus thuringiensis	IAM 12077	D16281.1
Bacillus xiamenensis	MCCC 1A00008	NR_148244.1
Brevibacillus borstelensis	DSM 6347	AB112721
Brevibacillus brevis	NBRC 15304	NR_041524.1
Brevibacillus centrosporus	NRRL NRS-664	NR_043414.1
Brevibacillus formosus	DSM 9885	AB112712.1
Brevibacillus laterosporus	IAM 12465	D16271
Lysinibacillus fusiformis	DSM 2898	AJ310083.1
Lysinibacillus sphaericus	ATCC 14577	NR_115724.1
Paenibacillus alvei	DSM 29	AJ320491
Paenibacillus amylolyticus	NRRL NRS-290	D85396.2
Paenibacillus apiarius	NRRL NRS-1438	NR_118834.1
Paenibacillus larvae subsp. *larvae*	ATCC 9545	NR_118956.1
Paenibacillus larvae subsp. *pulvifaciens*	ATCC 13537	KT363749.1
Paenibacillus macerans	IAM 12467	NR_040886
Paenibacillus polymyxa	DSM 36	AJ320493.1
Rummeliibacillus stabekisii	NBRC 104870	NR_114270
Micrococcus luteus (outlier)	DSM 20030	AJ536198.1

by using a Genova Nano spectrophotometer (JenWay). The purified PCR products of approximately 1,400 bp were sequenced by the dideoxy termination method by the commercial services of Macrogen Inc. (Seoul, Korea) or Unidad de Genómica, Instituto de Biotecnología, CICVYA - INTA (Hurlingham, Argentina). Sequence assembly and contig editing were performed by using CodonCode Aligner

Table 3
Colony appearance and growth of selected strains tested in HiCrome Bacillus agar.

Species	Strain/Isolate designation	Colonies in HiCrome Bacillus Agar	Ecometric Code	
			HiCrome Control	Bacillus medium
Bacillus amyloliquefaciens	m39		5	5
Bacillus badius	ATTC 14574		3.8	3.4
Bacillus cereus	ATCC 11778		5	5
Bacillus cereus	m388		5	5
Bacillus circulans	ATCC 4515		4	5
Bacillus clausii	FR231		1.6	5
Bacillus coagulans	NRRL NRS 609		5	5
Bacillus firmus	ATCC 8247		5	5
Bacillus licheniformis	NRRL B-1001		5	5
Bacillus megaterium	NRRL B-939		4.6	5
Bacillus mycoides	ATCC 10206		4	5

(*continued on next page*)

Table 3 (*continued*)

Species	Strain/Isolate designation	Colonies in HiCrome Bacillus Agar	Ecometric Code	
			HiCrome Control	Bacillus medium
Bacillus pumilus	ATCC 7061		5	5
Bacillus subtilis	m191		5	5
	NRRL B-543		4	5
Bacillus thuringiensis	ATCC 10792		5	5
Brevibacillus borstelensis	RC		4.2	5
Brevibacillus brevis	ATCC 8246		4.4	5
Brevibacillus laterosporus	CCT 0031		2.2	5
Lysinibacillus fusiformis	mv119		3.8	5
Lysinibacillus sphaericus	ATCC 245		1.4	5
Paenibacillus alvei	NRRL B-383		1	5
Paenibacillus amylolyticus	NRRL B-14940		4	5
Paenibacillus apiarius	ATCC 29575		4	5

Table 3 (*continued*)

Species		Strain/Isolate designation	Colonies in HiCrome Bacillus Agar	Ecometric Code	
				HiCrome Control	Bacillus medium
Paenibacillus larvae	ERIC I	ATCC 9545		2	5
	ERIC IV	ATCC 13537		2	5
	ERIC II	SAG 290		3.8	5
Paenibacillus polymyxa		NRRL B-510		5	5
Rummeliibacillus stabekisii		mv111		5	5

software (Codon Code Corporation, MA, USA). The partial sequences obtained were subjected to both Blast-N (http://www.ncbi.nlm.nih.gov), and EZBiocloud (http://www.ezbiocloud.n) search to identify sequences with the highest similarity by comparison only with sequences obtained from Type Cultures [2].

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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