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Paenibacillus melissococcoides sp. nov., isolated from a honey bee colony affected by European foulbrood disease

Florine Ory[1,*], Vincent Dietemann[1,2], Anne Guisolan[3], Ueli von Ah[3], Charlotte Fleuti[4], Simone Oberhaensli[5], Jean-Daniel Charrière[1] and Benjamin Dainat[1]

Abstract

A novel, facultatively anaerobic, Gram-stain-positive, motile, endospore-forming bacterium of the genus *Paenibacillus*, designated strain 2.1^T, was isolated from a colony of *Apis mellifera* affected by European foulbrood disease in Switzerland. The rod-shaped cells of strain 2.1^T were 2.2–6.5 µm long and 0.7–1.1 µm wide. Colonies of strain 2.1^T were orange-pigmented under oxic growth conditions on solid basal medium at 35–37 °C. Strain 2.1^T showed catalase and cytochrome c oxidase activity. Its polar lipid profile consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, aminophospholipid and phospholipid. The only respiratory quinone was menaquinone 7, and the major cellular fatty acids were anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$ and palmitic acid ($C_{16:0}$), which is consistent with other members of the genus *Paenibacillus*. The G+C content of the genomic DNA of strain 2.1^T was 53.3 mol%. Phylogenetic analyses based on the 16S rRNA gene sequence similarity showed that strain 2.1^T was closely related to *Paenibacillus dendritiformis* LMG 21716^T (99.7% similarity) and *Paenibacillus thiaminolyticus* DSM 7262^T (98.8%). The whole-genome average nucleotide identity between strain 2.1^T and the type strains of *P. dendritiformis* and *P. thiaminolyticus* was 92 and 91%, respectively, and thus lower than the 95% threshold value for delineation of genomic prokaryotic species. Based on the results of phylogenetic, genomic, phenotypic and chemotaxonomic analyses we propose the name *Paenibacillus melissococcoides* sp. nov. for this novel *Paenibacillus* species. The type strain is 2.1^T (=CCOS 2000^T=DSM 113619^T=LMG 32539^T).

DATA SUMMARY

Supplementary material and (Movie S1, available in the online version of this article) can be found on Figshare: https://doi.org/10.6084/m9.figshare.21679877.v1 [1]

INTRODUCTION

The genus *Paenibacillus* currently comprises more than 270 species with validly published and correct names [2]. *Paenibacillus* species are widely distributed in diverse environments, such as soil, plants, earthworms, humans and insects [3]. Among those found in insects, seven species have been associated with honey bees and three of these have been associated with honey bee diseases: *Paenibacillus larvae* is a well-described pathogen of honey bee brood, causing severe American foulbrood [4]. *Paenibacillus alvei* and *Paenibacillus dendritiformis* have been associated with another infectious brood disease, European foulbrood, caused by the bacterium *Melissococcus plutonius* [5]. Their pathogenicity and role in the course of European foulbrood disease have not been fully established. *P. alvei* alone caused mortality in honey bee larvae reared under laboratory conditions [6], but it

Author affiliations: [1]Swiss Bee Research Centre, Agroscope, Bern, Switzerland; [2]Department Ecology and Evolution, University of Lausanne, Lausanne, Switzerland; [3]Biotechnology Research Group, Agroscope, Bern, Switzerland; [4]Methods Development and Analytics, Agroscope, Bern, Switzerland; [5]Interfaculty Bioinformatics Unit and SIB Swiss Institute of Bioinformatics, University of Bern, Bern, Switzerland.
***Correspondence:** Florine Ory, oryflorine@gmail.com
Keywords: *Apis mellifera*; European foulbrood; honey bee; *Paenibacillus melissococcoides*.
Abbreviations: ANI, average nucleotide identity; BM, basal medium; CA, Caso agar; CDS, coding sequence; NA, nutrient agar.
The DDBJ/ENA/GenBank accession numbers for the whole genome sequences of *Paenibacillus melissococcoides* strains 2.1^T, 1.2 and 3.2 are, respectively, GCA_944800085, GCA_945318265 and GCA_945318285; and for the 16S rRNA sequences OW961637, OW961636 and OW961638.
The DDBJ/ENA/GenBank accession numbers for the whole genome sequences of *Paenibacillus dendritiformis* LMG 21716^T and *Paenibacillus thiaminolyticus* DSM 7262^T are GCA_945605565 and GCA_945318275, respectively. The genomic material of this study can be found under BioProject PRJEB49674.
Six supplementary figures and one movie are available with the online version of this article.

is unknown whether it can cause a disease at the colony level on its own. *P. dendritiformis* was isolated from diseased colonies, but its pathogenicity in honey bee brood has not yet been tested [7]. For these reasons, these bacteria are described as secondary invaders following *M. plutonius* infections. Other members of the genus *Paenibacillus*, *Paenibacillus apiarius* and *Paenibacillus thiaminolyticus*, were isolated from healthy honey bees and hive material [8, 9], and recently, *Paenibacillus apis* and *Paenibacillus intestini* were isolated from honey bee intestines [10].

The novel candidate strains of the genus *Paenibacillus* described in this report were isolated from a colony of the honey bee *Apis mellifera* showing symptoms of European foulbrood in Switzerland. They were designated as strains 2.1^T, 1.2 and 3.2. The novel strains are closely related to *P. dendritiformis* T168^T (=LMG 21716^T) [11] and *P. thiaminolyticus* JCM 8360^T (=DSM 7262^T) [9, 12] and are proposed to represent a new species with the name *Paenibacillus melissococcoides* sp. nov.

ISOLATION AND ECOLOGY

In June 2020, strains 2.1^T, 1.2 and 3.2 were isolated from a European foulbrood diseased colony of *Apis mellifera* that showed European foulbrood symptoms and tested positive for *M. plutonius*. The colony was located in the town of Reutigen (46° 41′ N 7° 37′ E, Bern, Switzerland). Droplets of the nutritive jelly given to worker larvae by nurse bees were sampled from several brood cells, diluted into sterile saline buffer, plated on basal medium (BM) agar specifically used for *M. plutonius* growth [13], and incubated for four days at 36 °C under anoxic conditions. Several colonies not conforming to the morphology of *M. plutonius* were observed on the plates. Three such colonies were picked and each was transferred into BM broth for growth at 36 °C in oxic conditions. Aliquots of culture were supplemented with 20% glycerol for long-term storage at −80 °C before phenotypic and phylogenetic-based taxonomic analyses.

16S RNA GENE PHYLOGENY

To perform the phylogenetic studies, the genomic DNA of strains 2.1^T, 1.2 and 3.2 were sequenced using the long-read technology of Pacific Bioscience (PacBio). High molecular weight genomic DNA was recovered using the guanidium thiocyanate method of DNA extraction [14]. DNA was extracted from single colonies grown on solid BM at 36 °C under oxic conditions. Multiplexed SMRTbell libraries were prepared for sequencing on the Sequel apparatus exactly according to the PacBio guidelines (Procedure and Checklist – Preparing Multiplexed Microbial Libraries Using SMRTbell Express Template Prep Kit 2.0; Part Number 101-696-100; version 06; March 2020). Data were assembled *de novo* using the microbial assembly pipeline (SMRTlink version 9.0) with default parameter settings. The sequences of strains 2.1^T, 1.2 and 3.2 were found to be identical (100% nucleotide similarity). Eight full-length 16S rRNA gene sequences were assembled for each strain. Seven of the 16S rRNA copies differed by single nucleotide polymorphisms at nine out of 1551 nucleotide sites. For further analysis, the full-length 16S rRNA gene sequence that was present in two identical copies was selected (accession numbers: 2.1^T, OW961637.1; 1.2, OW961636.1; and 3.2, OW961638.1). The full-length 16S rRNA gene sequence of strain 2.1^T was subjected to a BLAST search against the EzTaxon webserver (www.ezbiocloud.net). The highest pairwise sequence similarity was detected to the 16S rRNA gene of *P. dendritiformis* (99.7%), followed by *P. thiaminolyticus* (98.8%), *Paenibacillus popilliae* (98.2%) and *Paenibacillus lentimorbus* (98.1%).

To determine the phylogenetic relationships of strains 2.1^T, 1.2 and 3.2 within the genus *Paenibacillus*, the 16S rRNA gene sequences from each strain were used to generate an alignment (MUSCLE version 3.8.1551 using default parameter settings [15]) with the 236 *Paenibacillus* strains (235 species with two *P. polymyxa* strains) that have validly published and correct names [2] and high-quality 16S rRNA gene sequences [16]. *Bacillus pumilus* was used as an outgroup. The maximum-likelihood tree was calculated using IQ-TREE (multicore version 1.6.12) with parameter setting '-m TEST -bb 1000 -alrt 1000' on the IQ-TREE webserver [17–19]. The tree (Fig.S1) was visualised using the iTOL webserver [20].

For a better overview of the position of the three strains with the genus, a smaller phylogenetic tree was built based on the same method. The tree comprised honey bee-associated *Paenibacillus* species as well as the most ancestral species of each cluster (Fig. 1). In line with the BLAST search, the closest neighbours of strains 2.1^T, 1.2 and 3.2 were *P. dendritiformis*, *P. thiaminolyticus*, *P. popilliae* and *P. lentimorbus*. Given that *P. dendritiformis* and *P. thiaminolyticus* were previously reported as being associated with honey bees [7, 9], they were chosen for further comparative analyses.

GENOME FEATURES

To perform comparative analyses between strains 2.1^T, 1.2, 3.2 and related species, the whole genomes of *P. dendritiformis* LMG 21716^T and *P. thiaminolyticus* DSM 7262^T type strains (provided by BCCM LMG and DSMZ GmbH, respectively), were sequenced using the long-read technology of Pacific Biosciences, as previously described. Assembly statistics of strains 2.1^T, 1.2 and 3.2 are listed in Table 1. The levels of pairwise genome-based similarity were evaluated based on average nucleotide identity (ANI) values determined by FastANI [21]. The ANI values between strains 2.1^T, 1.2 and 3.2 were >99.9% (Table 2), confirming that they belonged to the same species, *P. melissococcoides* sp. nov. The ANI values of strains 2.1^T, 1.2 and 3.2 versus the closely

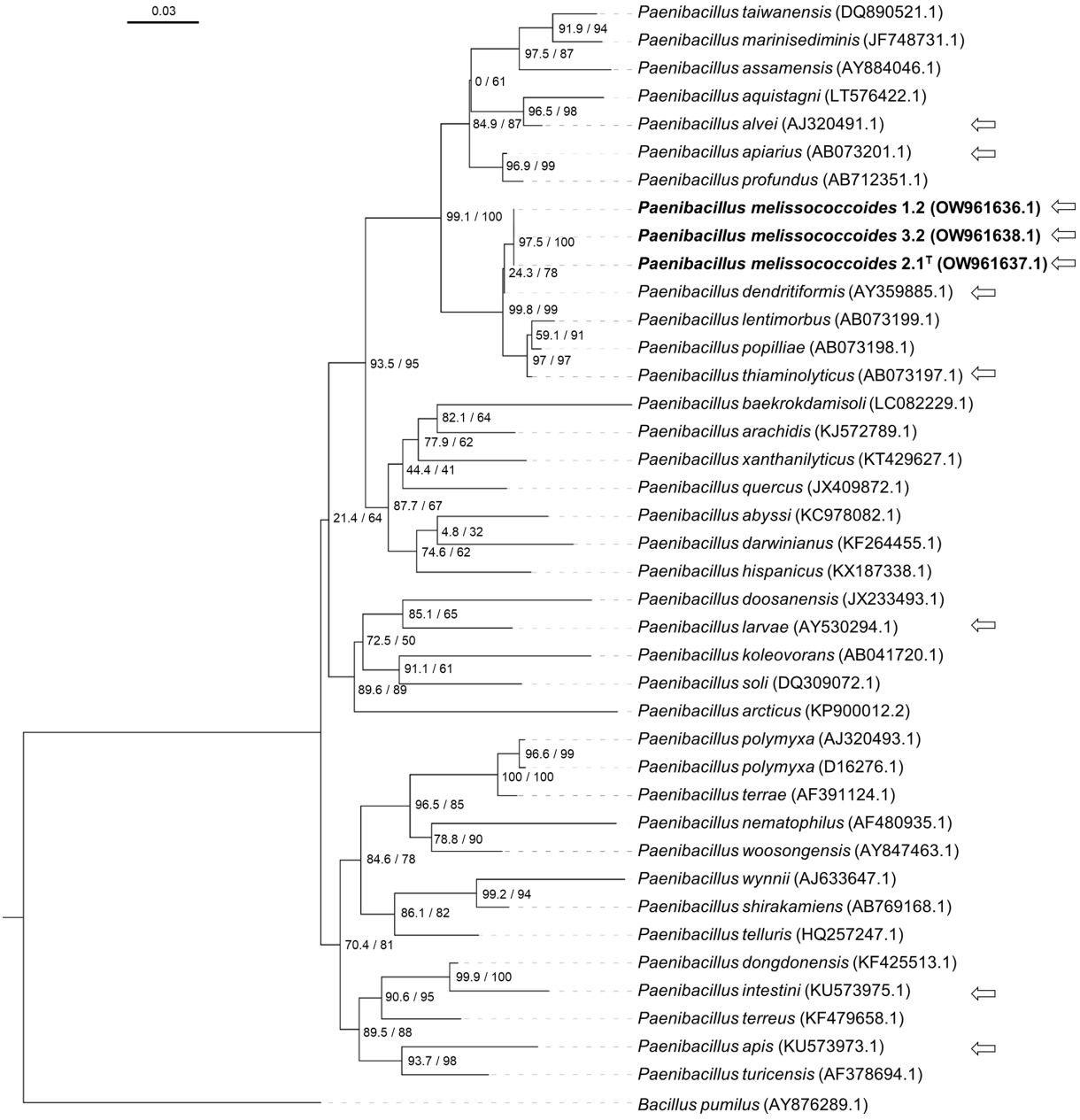

Fig. 1. Maximum-likelihood phylogenetic tree of 16S rRNA complete sequences showing the position of strains 2.1^T, 1.2 and 3.2 (in bold) with respect to the most ancestral species of each cluster of the genus *Paenibacillus* (Fig. S1). *Bacillus pumilus* was used as an outgroup. Node labels represent branch support values from SH-aLRT and ultrafast bootstrap (UFBoot) analyses. Arrows designate *Paenibacillus* species associated with honey bees. Bar, 0.03 substitutions per site.

related species *P. dendritiformis* LMG 21716^T and *P. thiaminolyticus* DSM 7262^T were <92.4% and <91.1%, respectively (Table 2). These differences are lower than the cut-off value of 95% for species-level differentiation [22, 23].

PHYSIOLOGY AND CHEMOTAXONOMY

To describe the physiology and chemotaxonomy of strains 2.1^T, 1.2 and 3.2, we studied their growth characteristics and conducted morphological and phenotypic analyses. All quantitative data presented in this section were averages generated from experiments run in duplicate. The growth abilities of the strains were tested using various growth media and compared to those of *P. dendritiformis* LMG 21716^T and *P. thiaminolyticus* DSM 7262^T. The following growth media were laboratory-prepared and tested: BM agar, nutrient agar (NA), caso agar (CA) and MYPGP agar commonly used for culture of respectively, *M. plutonius* [13],

Table 1. Whole genome information of the three strains of *Paenibacillus melissococcoides* sp. nov

Strain	DDBJ/EMBL/ GenBank accession no.	Assembly length (bp)	N50 (bp)	CDS	rRNAs (5S,16S,23S)	tRNAs	Contig no.	G+C content (mol%)	Plasmid no.
2.1^T	GCA_944800085	7186093	594566	7076	8, 8, 8	85	21	53.3	2
1.2	GCA_945318265	6765306	602769	6543	8, 8, 8	87	18	53.5	2
3.2	GCA_945318285	6837494	601760	6594	8, 8, 8	87	17	53.3	2

Table 2. Average nucleotide identity (ANI) values obtained comparing the whole genomes of *Paenibacillus melissococcoides* sp. nov. strains 2.1^T, 1.2, and 3.2 with closest related reference species *Paenibacillus dendritiformis* LMG 21716^T and *Paenibacillus thiaminolyticus* DSM 7262^T

Query	DDBJ/EMBL/GenBank accession no.	Reference	DDBJ/EMBL/GenBank accession no.	ANI (%)
2.1^T	GCA_944800085	1.2	GCA_945318265	99.99
1.2	GCA_945318265	3.2	GCA_945318285	99.99
3.2	GCA_945318285	2.1^T	GCA_944800085	99.99
2.1^T	GCA_944800085	LMG 21716^T	GCA_945605565	92.34
2.1^T	GCA_944800085	DSM 7262^T	GCA_945318275	91.09
1.2	GCA_945318265	LMG 21716^T	GCA_945605565	92.36
1.2	GCA_945318265	DSM 7262^T	GCA_945318275	91.05
3.2	GCA_945318285	LMG 21716^T	GCA_945605565	92.28
3.2	GCA_945318285	DSM 7262^T	GCA_945318275	91.03

P. thiaminolyticus [12], *P. dendritiformis* [11] and *P. larvae* [24]. The plates were inoculated with 50 µl sterile saline buffer (0.9% NaCl) containing 1500 CFU ml^{-1}, and incubated at 36 °C under oxic conditions. Strains 2.1^T, 1.2, 3.2, LMG 21716^T and DSM 7262^T were all able to grow on all the tested media (Fig. 2). Colonies of the five strains were visible 48 h post-inoculation. As colony morphology on NA and CA was similar, only the results on NA are shown. The colonies of strains 2.1^T, 1.2 and 3.2 were of two

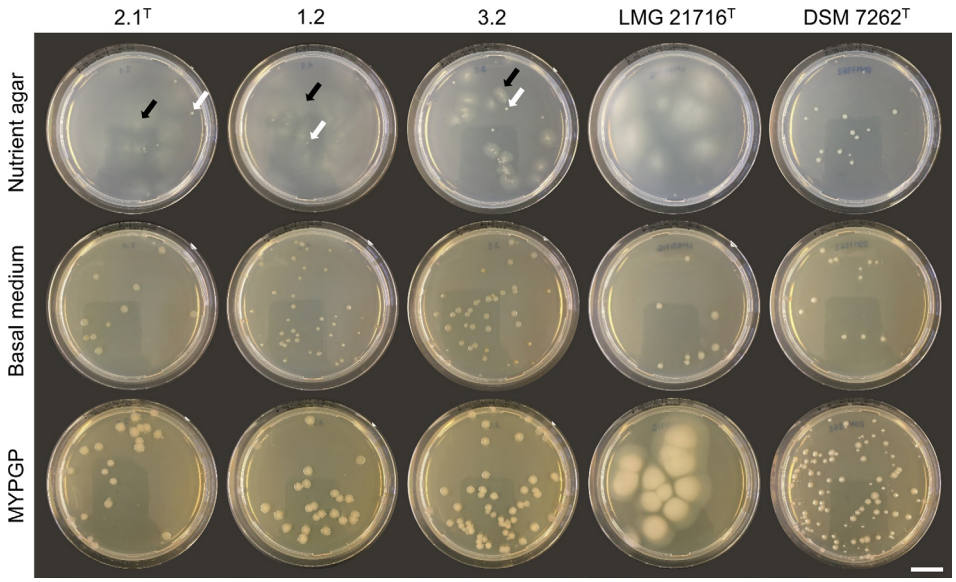

Fig. 2. Colony morphology of the three strains of *Paenibacillus melissococcoides* sp. nov. isolated from a European foulbrood diseased honey bee colony, of *Paenibacillus dendritiformis* LMG 21716^T and *Paenibacillus thiaminolyticus* DSM 7262^T after 48 h culture at 36 °C under oxic conditions on nutrient, basal medium, and MYPGP agar. White arrows point to colonies of the circular morphotype. Black arrows indicate colonies of the spread morphotype. Bar, 2 cm.

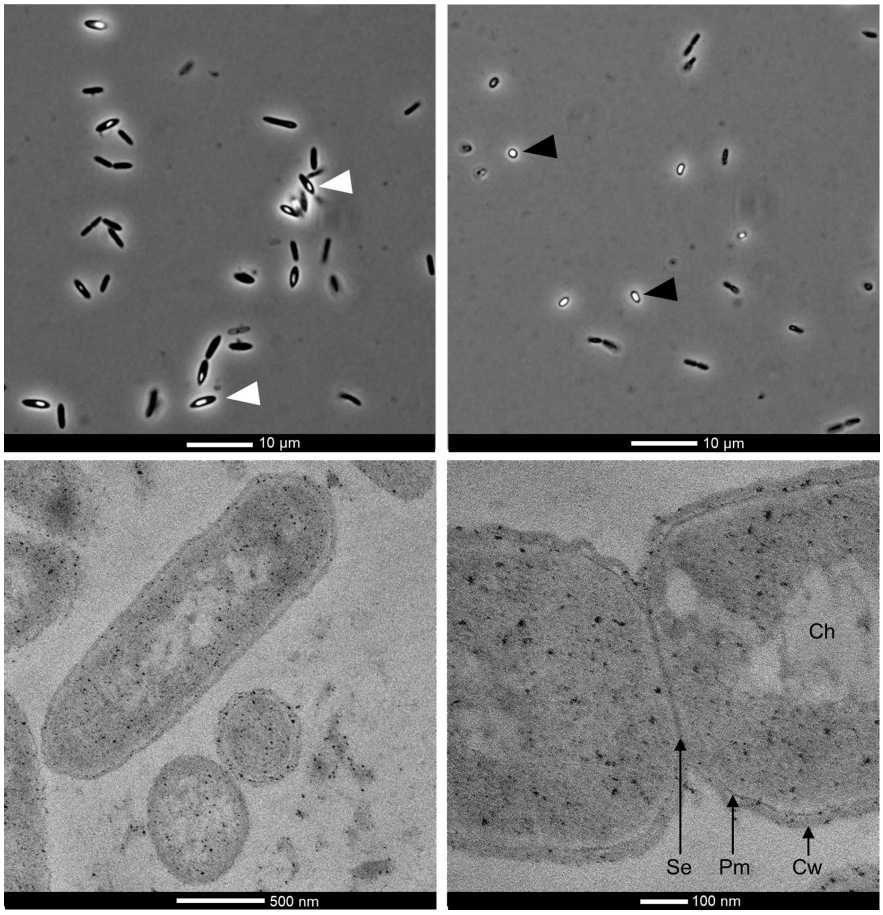

Fig. 3. Microscopic images of *Paenibacillus melissococcoides* sp. nov. strain 2.1^T cells. Top: phase-contrast microscopic images of endospore-forming cells (white arrowheads) and spores (black arrowheads). Bottom left: electron microscopic images of the rod-shaped cells. Bottom right: close up on the connection between two rods. Ch, chromosome. Se, septum. Pm, plasma membrane. Cw, cell wall.

morphotypes (spread and circular) on NA; however, only the circular morphotype was observed on BM and MYPGP. On BM, the colonies were circular with slightly undulate margins, umbonate, smooth and light white translucent, with an orange-pigmented centre. Pigmentation was not observed in the *P. dendritiformis* LMG 21716^T and *P. thiaminolyticus* DSM 7262^T colonies (Fig. S2). Colonies of *P. dendritiformis* LMG 21716^T were of the spread type on NA and MYPGP, but on BM they were circular. Colonies of *P. thiaminolyticus* DSM 7262^T were circular on all tested media. BM was selected for all further experiments, because it was the only medium on which the five strains showed a circular morphology and thus provided the most comparable conditions.

Experiments to determine the NaCl, temperature and oxygen requirements allowing for *P. melissococcoides* sp. nov. growth were performed using culture tubes filled with 4 ml BM broth and inoculated with strain 2.1^T. Bacterial inoculum consisted of 10 single CFUs picked on BM agar, and homogenised into 1 ml sterile saline buffer. Ten microlitres of this bacterial solution were used to inoculate the BM broth. NaCl sensitivity was tested under oxic conditions at 37 °C in the range of 0–15%(0, 1, 3, 5, 7, 10 and 15% w/v). Temperature sensitivity was tested under oxic conditions in the range of 4–50 °C (4, 10, 15, 20, 25, 30, 35, 37, 42 and 50 °C). Oxygen requirement was tested at 37 °C under oxic or anoxic conditions (>15% CO_2 atmosphere, GENanaer, bioMérieux). The absorbance value (turbidity) of the culture medium was used as a metric for the growth rate. Absorbance was measured using a SpectraMax QuickDrop (Molecular Devices) based on the optical density at 600 nm after 24, 48, 72 and 168 h of incubation. The temperature and NaCl ranges for growth of strain 2.1^T were 25–42 °C (optimum, 35–42 °C) and 0–1% NaCl (optimum, 0% NaCl), respectively. Strain 2.1^T grew under oxic and anoxic conditions (optimum, oxic) (Fig. S3). All subsequent analyses were performed with bacteria grown on BM agar under optimal conditions, i.e., at 36±1 °C, without NaCl and under oxic conditions.

Gram staining was performed as described in [25]. Strain 2.1^T was grown under optimal conditions for 18 and 36 h, after which single colonies were picked and suspended in 100 µl sterile saline solution (0.9% NaCl). Ten microlitres of the bacterial suspension were smeared and heat-fixed on microscope slide grids prior to staining. Cell size and spore formation were investigated using a phase-contrast Olympus BX41 microscope. Vegetative cell size was evaluated from 18-h-old cultures. Endospore formation

Table 3. Biochemical characteristics of *Paenibacillus melissococcoides* sp. nov. strains 2.1^T, 1.2 and 3.2 in comparison with closely related *Paenibacillus dendritiformis* LMG 21716^T and *Paenibacillus thiaminolyticus* DSM 7262^T

Strains: 1, *P. melissococcoides* sp. nov. 2.1^T; 2. *P. melissococcoides* sp. nov. 1.2; 3, *P. melissococcoides* sp. nov. 3.2; 4, *P. dendritiformis* LMG 21716^T; 5, *P. thiaminolyticus* DSM 7262^T. All strains and species were analysed under the same laboratory conditions. Symbols: +, positive reaction; w, weak positive reaction; –, negative reaction. Characteristics distinguishing the different *Paenibacillus* strains and species are indicated in bold.

Characteristics	**1**	**2**	**3**	**4**	**5**
Catalase	+	+	+	+	+
Oxidase	**+**	**+**	**+**	**–**	**–**
Acid formation from:					
Glycerol	**–**	**–**	**–**	**+**	**+**
Erythritol	–	–	–	–	–
Arabinose	–	–	–	–	–
L-Arabinose	–	–	–	–	–
D-Ribose	+	w	w	+	+
Xylose	–	–	–	–	–
L-Xylose	–	–	–	–	–
Adonitol	–	–	–	+	–
Methyl β-D-xylopyranoside	–	–	–	–	–
Galactose	+	+	+	w	+
Glucose	+	+	+	+	+
Fructose	–	–	–	w	+
Mannose	**–**	**–**	**–**	**+**	**+**
L-Sorbose	–	–	–	–	–
L-Rhamnose	–	–	–	–	–
Dulcitol	–	–	–	–	–
Inositol	–	–	–	–	–
D-Mannitol	–	–	–	–	–
Sorbitol	–	–	–	–	–
Methyl α-D-mannopyranoside	w	w	w	w	+
Methyl α-D-glucopyranoside	+	w	w	+	+
N-Acetylglucosamine	w	w	w	+	+
Amygdalin	+	+	+	+	+
Arbutin	+	+	+	+	+
Aesculin	+	+	+	+	+
Salicin	+	w	+	+	+
Cellobiose	w	w	w	+	+
Maltose	+	+	+	+	+
Lactose	w	w	w	w	+
Melibiose	+	+	+	+	+
Sucrose	+	+	+	+	+
Trehalose	+	+	+	+	+

Continued

Table 3. Continued

Characteristics	1	2	3	4	5
Inulin	w	w	w	–	–
Melezitose	w	w	w	+	+
Raffinose	+	+	+	+	+
Starch	+	+	+	+	+
Glycogen	w	w	w	–	+
Xylitol	w	w	w	–	–
Gentiobiose	+	+	+	+	+
Turanose	+	w	+	+	+
Lyxose	–	–	–	–	–
Tagatose	–	–	–	–	–
Fucose	–	–	–	–	–
L-Fucose	–	–	–	–	–
Arabitol	–	–	–	–	–
L-Arabitol	–	–	–	–	–
Potassium gluconate	+	w	w	w	w
Potassium 2-ketogluconate	–	–	–	–	–
Potassium 5-ketogluconate	–	–	–	–	–

was observed in cultures incubated for 36 h under optimal conditions, and stressed at 4 °C for six days. Spore size was measured from cultures incubated for 36 h under optimal conditions and then at 20 °C for six days. The cell morphology of strain 2.1^T was analysed using transmission electron microscopy according to [26] and adapted to bacteria. The bacteria were grown on BM at 25 °C for 72–120 h. Visible colonies were suspended and fixed for 1 h in 2.5% glutaraldehyde containing PIPES buffer (0.07 M, pH 7.0). Ultra-thin sections on microscopic grids were stained with 2% acetate uranyl and observed under a transmission electron microscope Tecnai G2 Spirit (FEI). The motility of strains 2.1^T, 1.2 and 3.2 was tested by the hanging-drop method [27], and on semi-solid BM 0.4% agar [28]. *P. melissococcoides* sp. nov. cells stained Gram-positive after 18 h of culture and Gram-negative after 36 h of culture (Fig. S4), suggesting they possess a thick peptidoglycan wall structure, which can be damaged over culture time. Cells were motile (Movie S1, Fig. S5), rod-shaped, 2.2–6.5 µm long and 0.7–1.1 µm wide. They often formed dyads connected by a septum. They formed central or subterminal endospores that were oval-shaped, 0.5–2.0 µm long and 0.2–1.8 µm wide (Fig. 3).

For biochemical and metabolic characterization of strains 2.1^T, 1.2, 3.2, *P. dendritiformis* LMG 21716^T and *P. thiaminolyticus* DSM 7262^T, standard assays were performed (Table 3). Catalase activity was tested by the direct addition of a drop of 3% H_2O_2 on bacterial cells. The O_2 production and foaming reaction by the strains were interpreted as positive results for the catalase. The presence of cytochrome c oxidase was assessed by spreading bacteria on ashless paper (Schleicher and Schuell) with a drop of 1% dimethyl-*p*-phenylenediamine (bioMérieux) and observing whether a blue–violet colouration indicating oxidation by cytochrome c became visible. Strains 2.1^T, 1.2 and 3.2 were positive for catalase and cytochrome c oxidase, whereas LMG 21716^T and DSM 7262^T were only positive for catalase. To determine acid production from carbohydrate analysis, we used API 50 CH strips and API 50 CHE/B Medium (bioMérieux) according to the manufacturer's recommendations. The positive oxidase activity and the absence of acid formation from glycerol and mannose in *P. melissococcoides* sp. nov. were the major biochemical differences distinguishing the strains from closely related *P. dendritiformis* LMG 21716^T and *P. thiaminolyticus* DSM 7262^T (Table 3).

Cellular fatty acid profiles of *P. melissococcoides* sp. nov. strains 2.1^T, 1.2, 3.2, *P. dendritiformis* LMG 21716^T and *P. thiaminolyticus* DSM 7262^T were determined by high-resolution lipid analysis using a gas chromatograph with a flame ionization detector (GC-FID; 6890+, Agilent Technologies) and compared with previously published profiles of two other members of the genus *Paenibacillus*, *P. alvei* IFO 3343^T and *P. apiarius* NRRL NRS-1438^T [9]. The bacteria were grown on BM under optimal conditions. After 48 h culture, bacteria were harvested from the medium using a sterile inoculation loop and lyophilized using a vacuum controller V-855 coupled with a vacuum pump V-700 (Buchi) and a concentrator plus (Eppendorf). Approximately 10 mg dry bacterial material was prepared for analysis by adding 2 µl of the internal standard methyl nonanoate (C9, 0.007 µg µl^{-1}) for quantification. Transesterification was initiated by adding 125 µl of KOH/MeOH (2 mol l^{-1}) to the samples. After 5 min, 0.3 g of

Table 4. Cellular fatty acid profiles of *Paenibacillus melissococcoides* sp. nov. strains 2.1^T, 1.2 and 3.2 in comparison with those of closely related members of the genus *Paenibacillus*

Strains: 1, 2.1^T; 2, 1.2; 3, 3.2; 4, *P. dendritiformis* LMG 21716^T; 5, *P. thiaminolyticus* DSM 7262^T; 6, *P. alvei* IFO 3343^T; 7, *P. apiarius* NRRL NRS-1438^T. Data for strains 1–5 were investigated in this study under the same laboratory conditions; data for strains 6 and 7 were obtained from [9]. NA, Data not available. Fatty acid content distinguishing the different *Paenibacillus* strains is indicated in bold.

Fatty acid (%)	1	2	3	4	5	6	7
iso-$C_{14:0}$	0.6	0.7	0.6	0.4	1.6	0.3	0.2
$C_{14:0}$	1.2	1.3	1.3	1.5	2.1	2.3	0.6
iso-$C_{15:0}$	8.7	9.0	8.7	6.5	7.4	12.3	8.3
anteiso-$C_{15:0}$	37.9	37.6	37.4	43.6	38.4	53.9	60.5
$C_{15:0}$	2.2	2.5	2.3	1.4	1.3	1.6	2.2
iso-$C_{16:0}$	4.8	5.2	5.0	4.3	10.6	2.3	4.2
$C_{16:0}$	**6.7**	**6.7**	**7.1**	**16.9**	**16.1**	**15.3**	**4.8**
iso-$C_{17:0}$	7.7	7.8	7.9	8.0	6.9	3.3	5.5
anteiso-$C_{17:0}$	**27.2**	**26.2**	**26.7**	**15.1**	**12.5**	**3.3**	**16.3**
$C_{17:0}$	1.1	1.2	1.2	0.6	0.6	NA	NA
iso-$C_{18:0}$	0.2	0.2	0.2	0.1	0.3	NA	NA
$C_{18:0}$	0.3	0.3	0.3	0.2	0.4	NA	NA
Other peaks	1.3	1.4	1.3	1.2	1.7	NA	NA

sodium hydrogen sulphate monohydrate was added to stop the reaction. Fatty acid extraction was performed by adding 100 µl of hexane to the samples, which were vortexed and centrifuged. The supernatant (5 µl) containing the fatty acids was injected into the GC-FID column for analysis. The temperature ramp was as follows: 5 min at 60 °C, from 60 to 165 °C at 14 °C per min, 1 min at 165 °C, from 165 to 225 °C at 12 °C per min and 17 min at 225 °C. The chromatograms were integrated using Agilent software (ChemStation). The most abundant cellular fatty acids of strains 2.1^T, 1.2 and 3.2 were anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$ and palmitic acid ($C_{16:0}$). The same pattern was observed for *P. dendritiformis* LMG 21716^T and *P. thiaminolyticus* DSM 7262^T, except that the proportions shifted towards markedly larger amounts of palmitic acid ($C_{16:0}$) and smaller amounts of anteiso-$C_{17:0}$. Previously published cellular fatty acid profiles of members of the genus *Paenibacillus* showed similar results [9], with anteiso-$C_{15:0}$ as a major cellular fatty acid (Table 4). Overall, strains 2.1^T, 1.2 and 2.3 could be distinguished from other members of *Paenibacillus* by larger amounts of anteiso-$C_{17:0}$, and lower amount of palmitic acid ($C_{16:0}$).

Analysis of polar lipid profiles, respiratory quinones and peptidoglycans was carried out by DSMZ Services, Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. The polar lipid profiles consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, aminophospholipid and phospholipid (Fig. S6). The only respiratory quinone was menaquinone 7. The peptidoglycan type was isolated and studied following published protocols [29]. The total hydrolysate (100 °C, 4 N HCl, 16 h) of the peptidoglycan contained muramic acid (Mur) and the amino acids diaminopimelic acid (Dpm), alanine (Ala) and glutamic acid (Glu). Quantification of amino acids by GC/MS of *N*-heptafluorobutyric amino acid isobutylesters resulted in the following molar ratio: 1.7 Ala : 1.0 Glu:0.5 Dpm. The identity of all amino acids was confirmed by agreement in the gas-chromatographic retention time with those of authentic standards and by characteristic mass spectrometric fragment ions of the derivatives. The peptides M-Ala, Ala-Glu, Ala-Glu-Dpm and Dpm-Ala-Dpm were detected after hydrolysis under milder conditions (4 N HCl, 45 min, 100 °C). From these data, the occurrence of the peptidoglycan type A1γ meso-Dpm-direct was concluded.

DESCRIPTION OF *PAENIBACILLUS MELISSOCOCCOIDES* SP. NOV.

Paenibacillus melissococcoides [me.lis.so.coc.co'i.des; N.L. masc. n. *Melissococcus,* a bacterial genus; L. adj. suff. *-oides*, resembling, similar (from Gr. neut. adj. suff. *-eides* resembling, similar; Gr. neut. n. *eidos*, that which is seen, form, shape, figure); N.L. masc. adj. *melissococcoides*, *Melissococcus*-like].

Cells of this Gram-stain positive bacterium are motile, rod-shaped, 2.2–6.5 µm long and 0.7–1.1 µm wide, and form central or subterminal endospores that are oval in shape. Growth on basal medium broth occurs from 25 to 42 °C, in 0–1% NaCl concentration at 37 °C and under oxic or anoxic conditions. Optimal growth on BM agar occurs under oxic condition at 35–37 °C with

0% NaCl. After 48 h culture, colonies are circular (1–5 mm diameter), with slightly undulate margins, umbonate, smooth and light white translucent, with an orange-pigmented centre. Positive for catalase and cytochrome c oxidase. Acid formation from galactose, glucose, amygdalin, arbutin, aesculin, salicin, maltose, melibiose, sucrose, trehalose, raffinose, starch, gentiobiose and turanose is observed. Weak formation of acid from D-ribose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, cellobiose, lactose, inulin, melezitose, glycogen xylitol and potassium gluconate is detected. The most abundant cellular fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$ and palmitic acid ($C_{16:0}$). The polar lipid profile consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, aminophospholipid and phospholipid. The only respiratory quinone is menaquinone 7. The peptidoglycan type is A1γ *meso*-Dpm-direct.

The type strain is 2.1^T (=CCOS 2000^T=DSM 113619^T=LMG 32539^T), isolated from a colony of honey bees, *Apis mellifera*, presenting symptoms of European foulbrood in Switzerland, canton of Bern. The genomic DNA G+C content of the type strain is 53.3 mol%.

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Author contribution
F. O.: conceptualization, investigation, formal analysis, validation, visualization, writing – original. V. D.: conceptualization, supervision, project administration, validation, visualization, writing – review and editing. A.G: investigation, formal analysis. U.v.A.: investigation, formal analysis. C.F.R.: investigation, formal analysis, writing – review and editing S.O.R: investigation, formal analysis, writing – review and editing. J.D.C.: project administration, validation, writing – review and editing. B. D.: conceptualization, investigation, supervision, project administration, validation, visualization, writing – review and editing.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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