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Bacillus onubensis sp. nov., isolated from the air of two Andalusian caves

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Abstract: Two Gram-positive, catalase-positive, oxidase-negative, motile, endospore-forming, rod-shaped bacteria, designated as 0911MAR22V3T and 0911TES10J4, were isolated from air samples collected in two show caves, located in Andalusia, Southern Spain. Phylogenetic analysis based on 16S rRNA gene sequences indicated that both strains were indistinguishable and they were most closely related to Bacillus humi DSM 16318T (98%).DNA-DNA hybridization values of the strain 0911MAR22V3T with respect to strain 0911TES10J4 and Bacillus humi DSM 16318T were 76.8% (73.9%, reciprocal) and 56.9% (63.3%, reciprocal analysis), respectively. Whole genome average nucleotide identity (ANI) values of both strains were in the threshold value for

species delineation and less than 85% with B. humi. Strains 0911MAR22V3T and 0911TES10J4 grew at 10-47°C (optimum 37°C), at pH 6-9.5 and with 0-8% (w/v) NaCl (optimum 1%). In both strains the dominant isoprenoid quinone was MK-7, the major cellular polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and two more phospholipids, the predominant fatty acids were iso-C15:0 and anteiso-C15:0 and the DNA G+C content was 38 mol%. On the basis of their phylogenetic relatedness and their phenotypic and genotypic features, the strains 0911MAR22V3T and 0911TES10J4 should be attributed to a novel species within the genus Bacillus, for which the name Bacillus onubensis sp. nov. is proposed. The type strain is 0911MAR22V3T (=LMG 27963T =CECT 8479T); and strain 0911TES10J4 (CECT 8478) is a reference strain.

Keywords: *Bacillus*; *Bacillus onubensis;* show cave; 16S rRNA gene sequence; polyphasic taxonomy

The GenBank/EMBL/DDBJ accession numbers for the genome and 16S rRNA gene sequences of 0911MAR22V3^T and 0911TES10J4 are NSEB00000000, LN650668, NSEA00000000 and LN774332.

Two Gram-positive, catalase-positive, oxidase-negative, motile, endospore-forming, rod-shaped bacteria, designated as 0911MAR22V3^T and 0911TES10J4, were isolated from air samples collected in two show caves, located in Andalusia, Southern Spain. Phylogenetic analysis based on 16S rRNA gene sequences indicated that both strains were indistinguishable and they were most closely related to *Bacillus humi* DSM 16318^T (98%). DNA-DNA

hybridization values of the strain 0911MAR22V3^T with respect to strain 0911TES10J4 and *Bacillus humi* DSM 16318^T were 76.8% (73.9%, reciprocal) and 56.9% (63.3%, reciprocal analysis), respectively. Whole genome average nucleotide identity (ANI) values of both strains were in the threshold value for species delineation and less than 85% with *B. humi.* Strains 0911MAR22V3^T and 0911TES10J4 grew at 10-47°C (optimum 37°C), at pH 6-9.5 and with 0-8% (w/v) NaCl (optimum 1%). In both strains the dominant isoprenoid quinone was MK-7, the major cellular polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and two more phospholipids, the predominant fatty acids were *iso*-$C_{15:0}$ and *anteiso*-$C_{15:0}$ and the DNA G+C content was 38 mol%. On the basis of their phylogenetic relatedness and their phenotypic and genotypic features, the strains 0911MAR22V3^T and 0911TES10J4 should be attributed to a novel species within the genus *Bacillus*, for which the name *Bacillus onubensis* sp. nov. is proposed. The type strain is 0911MAR22V3^T (=LMG 27963^T =CECT 8479^T); and strain 0911TES10J4 (CECT 8478) is a reference strain.

The genus *Bacillus* was described for the first time in 1872 by Cohn [5]. Along the last 146 years, a wide variety of species isolated from different environments and niches have been described. Members of the genus *Bacillus* show physiological versatility [22] and have been isolated from soils [11, 12,

17], marine environments [3, 39], plants [25, 43], foods [40], air [30] and humans [16].

The aim of the present work was to characterize and describe through a polyphasic approach the strains 0911MAR22V3^T and 0911TES10J4, isolated from air samples collected in two Andalusian show caves, Gruta de las Maravillas (Aracena, Huelva) and Cueva del Tesoro (Rincon de la Victoria, Malaga), respectively, located in Southern Spain. The distance between both caves is 318 km.

The strains were isolated on trypticase soy agar medium (TSA, BD) with cycloheximide (Applichem) (50 µg/mL).

For phylogenetic analysis of these strains, genomic DNA was extracted according to Marmur [23]. Amplification of 16S rRNA gene was performed following the method described by Laiz *et al*. [19]. The identification of phylogenetic neighbours was determined using the global alignment algorithm on the EzTaxon-e [41]. The 16S rRNA gene sequences of the strains 0911MAR22V3^T, 0911TES10J4, the closest related strains and the type strain of the genus, *Bacillus subtilis* DSM 10^T, were multiply aligned using MUSCLE [7]. Phylogenetic trees were constructed using the neighbour-joining [27], maximum-likelihood [8] and maximum-parsimony [15] methods in MEGA version 7.0 [18]. A bootstrap analysis of 1000 re-samplings was used to evaluate the trees robustness. On the basis of 16S rRNA gene sequence similarity comparisons, strains 0911MAR22V3^T and 0911TES10J4 were 100% identical among them. The maximum-likelihood analysis (Fig. 1) indicated that the closest relatives of both strains were *Bacillus humi* DSM 16318^T, *Bacillus*

galliciensis DSM 21539^T and *Bacillus luteolus* DSM 22388^T. These three species were selected as reference strains. In this study morphological, physiological, biochemical and chemotaxonomic characteristics were determined for strains 0911MAR22V3^T, 0911TES10J4 and the reference strains.

Comparative studies were carried out in triplicate on TSA plates at 30°C for all strains unless otherwise indicated. Gram-reaction was performed following the Hucker modification [6] and was confirmed by KOH-lysis test [10]. Cell size, morphology and motility of 2 days old cells grown on TSA plates were examined with a phase contrast microscope (Zeiss Axioskope 2 equipped with the image analysis software AxioVision Rel 4.8). Motility was tested on trypticase soy broth containing 0.3 % agar [33]. Endospores were detected by the application of the Wirtz-Conklin staining technique [29]. In addition, cells of strain 0911MAR22V3^T were observed by field emission scanning electron microscopy (FESEM). The strain was previously grown on TSA medium and incubated at 30ºC for 3 days. The resulting biomass was collected from the agar surface and fixed with 2.5% glutaraldehyde in 0.1M cacodylate-buffer (pH 7.4) at 4ºC for 2 h. The cells were separated from the mixture by centrifugation and washed twice in cacodylate-buffer for 5 min each and post-fixed in 1% osmium tetroxide for 1 h at 4ºC. After dehydration by subsequent dilution series in ethanol and acetone, the bacterium was dried in a critical point drying device (Leica EM 300) at 34.5ºC. Finally, the bacterial cells were sputter-coated with a thin gold/palladium film and examined in a Jeol JSM-7001F microscope. FESEM studies were carried out in a secondary electron detection mode with an acceleration potential of 15 kV. Morphologically, the strain 0911MAR22V3^T was

a rod-shaped bacterium in which endospore in swollen sporangia was observed (Fig. 2). Catalase activity was determined by the production of bubbles after addition of a drop of 3 % (v/v) hydrogen peroxide on cellular biomass smeared on a glass slide. Oxidase activity was tested using the BBL™DrySlide™ Oxidase (BD). The temperature range for growth was assessed at 4, 8, 10, 20, 25, 30, 37, 40, 47 and 50°C. Salt tolerance was tested in the presence of 0-10% (w/v) NaCl with increments of 1% on nutrient agar (BD). Growth at different pH values was determined on trypticase soy broth and agar plates adjusted to pH 5.0-12.0 (at intervals of 1.0 pH units) by using HCl 1 M and NaOH 1 M solutions. The pH values were verified after autoclaving. Anaerobic growth was detected in anaerobic chambers (GENbox anaer, bioMérieux). Physiological characteristics were determined with API 20NE, API ZYM and API 50CH galleries (bioMérieux), according to the manufacturer's instructions. Hydrolysis of Tweens 20, 40 and 80 were tested as described by Lányi [20]. Antibiotic susceptibility was estimated by the agar diffusion technique, using commercial antibiotic discs (BD). Cellular fatty acid profiles were analyzed in triplicate after collecting biomass from a culture grown for 3 days on TSA plates at 30°C following the methodology described by Jurado *et al*. [14]. G+C content of genomic DNA, analysis of respiratory quinones and polar lipids composition were carried out by the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). The DNA G+C content were analysed by HPLC [32] and values were calculated by the methodology of Mesbah *et al*. [24]. Respiratory lipoquinones were extracted following the method described by Tindall [34, 35], separated by thin layer chromatography on silica gel and analysed by HPLC. Polar lipids were extracted (modified after

Bligh and Dyer, [4]), separated by two dimensional silica gel thin layer chromatography and specific functional groups were detected according to Tindall et al. [36]. DNA-DNA hybridization (DDH) of the strain 0911MAR22V3^T with the strains 0911TES10J4 and *Bacillus humi* DSM 16318^T was performed according to Urdiain *et al*. [37]. Genomic fingerprinting profiles of the strain 0911MAR22V3^T, 0911TES10J4 and the closest related type strains were obtained by random amplified polymorphic DNA (RAPD) method using the primer M13 (5'-GAG GGT GGC GGT TCT-3') [13] and following the polymerase chain reaction (PCR) conditions described by Giraffa *et al*. [9]. PCR products were visualized by electrophoresis (60 V, 2 h) in agarose gel 1.5 % (w/v), using SYBR GREEN (Molecular Probes) as nucleic acid stain and Easy Ladder I (Bioline) as DNA molecular weight marker.

The draft genome sequence of the strains 0911MAR22V3^T and 0911TES10J4 were sequenced using the Illumina HiSeq 2500 platform (2 X 100-bp paired-end) by STAB VIDA (Lisbon, Portugal). The analysis of the generated raw sequence data was carried out using GLC Genomics Workbench 10.1.1 and RAST [1]. The genome assembly was performed using the algorithm package Velvet version 1.2.10 [42] based on the Brujin graphs. Genome annotation was performed by RAST [1]. The subsequent assembly for the strain 0911MAR22V3^T generated 39 contigs and the total genome size of 4,849,192 bp; and for the strain 0911TES10J4, 48 contigs and the total genome size of 4,722,324 bp. The genome sequence accession numbers for 0911MAR22V3^T, 0911TES10J4, the closest species to both strains, *Bacillus humi* DSM 16318^T and the type species of the genus, *Bacillus subtilis* strain 168 are NSEB00000000, NSEA00000000, LMBW00000000 and NC_000964.3.

Calculations of average nucleotide identity (ANI) were performed between genome sequences of strains 0911MAR22V3^T and 0911TES10J4, *B. humi* and *B. subtilis*. Calculations of ANIb and ANIm according to BLAST and MUMmer algorithms, and TETRA values were carried out using the JSpecies software [26] whereas OrthoANI measurements were calculated as described Lee et al. [21].

The strains 0911MAR22V3^T and 0911TES10J4 were Gram positive, motile, endospore-forming, catalase positive and presented menaquinone-7 as the predominant isoprenoid quinone. Moreover, these strains showed differences with respect to the reference strains in fatty acid proportion (Supplementary Table S1), growth conditions, physiological and biochemical characteristics, enzymatic activities, hydrolysis capability and antibiotic susceptibility (Table 1). Both strains differed from the reference strains in their ability to grow at 47°C, hydrolysis of Tween 40 and 80, alkaline phosphatase and trypsin activities, assimilation of glucose and acid production from L-arabitol, glycogen, D-tagatose and potassium 5-ketogluconate. Strains 0911MAR22V3^T and 0911TES10J4 showed some differences, such as in their ability to grow under anaerobic conditions; cysteine arylamidase and trypsin activities; assimilation of arabinose; acid production from D-adonitol, amydalin, D-arabinose, L-arabinose, D-cellobiose, glycerol, inositol, inulin, methyl-α-D-glucopyranoside, D-melibiose, potassium 5-ketogluconate, D-raffinose, D-ribose, salicin, D-tagatose, D-turanose and xylitol; and their susceptibility to fosfomycin+glucose-6-phosphate. Strain 0911TES10J4 contained an aminolipid in their membranes that was not present in 0911MAR22V3^T (Supplementary Fig. S1). The strain 0911MAR22V3^T showed shorter cells than 0911TES10J4 (2.8-3.8 µm and 4.0-

5.4 µm long, respectively) and smaller colonies (1-3 mm and 1-5 mm in diameter, respectively).

On the basis of the 16S rRNA gene sequences comparison, strains 0911MAR22V3^T and 0911TES10J4 showed a sequence similarity of 98% with *Bacillus humi* DSM 16318^T, 96% with *Bacillus galliciensis* DSM 21539^T, 95.7% with *Bacillus luteolus* DSM 22388^T and 94.5 % with *Bacillus subtilis* DSM 10^T. The strain 0911MAR22V3^T shared sequence similarities above 97% with the strains 0911TES10J4 and *Bacillus humi* DSM 16318^T, which is considered the reference value to define the bacterial species [31]. DNA-DNA hybridization was carried out between these strains to establish their relation at the species level. Strain 0911MAR22V3^T showed DNA-DNA relatedness of 76.8 % with strain 0911TES10J4 (73.9 % reciprocal analysis) and 56.9 % with *Bacillus humi* DSM 16318^T (63.3 %, reciprocal). Considering a re-association value of 70 % for the species circumscription [38], these results indicate that the strains 0911MAR22V3^T and 0911TES10J4 belong to the same species while they were not related to *Bacillus humi* DSM 16318^T at the species level. Genomic fingerprinting profiles (Supplementary Fig. S2) showed similar banding patterns between the strains 0911MAR22V3^T and 0911TES10J4, which differed with respect to the reference strains. In addition, other parameters (ANIb, ANIm, OrthoANI and TETRA values) described in Table 2 corroborate the data obtained by DDH and RAPD. The strains 0911MAR22V3^T and 0911TES10J4 showed ANI similarity and OrthoANI values ranging from 93.59 to 94.62% among them, and less than 85% with *B. humi* (Table 2). Values obtained from the three indexes were slightly lower (94-96%) than those proposed by Richter and Rossello [26]. These values are included in the transition zone described by

these authors confirming that the two strains belong to the same species, and should be classified as a novel species of the genus *Bacillus*. Moreover, the results of TETRA calculations show a high coefficient (>0.99) between the strains 0911MAR22V3^T and 0911TES10J4, and a low coefficient (<0.99) between *B. humi* and both strains supporting the species circumscription. Based on the results of ANIb, ANIm, OrthoANI calculations reinforced by high TETRA correlations values it can be concluded that the strains 0911MAR22V3^T and 0911TES10J4 should be classified as members of the same species.

Phylogenetic, phenotypic and genotypic data obtained suggest that the strains 0911MAR22V3^T and 0911TES10J4 represent a novel species within the genus *Bacillus*, for which the name *Bacillus onubensis* sp. nov. is proposed.

Description of *Bacillus onubensis* sp. nov.

Bacillus onubensis (o.nu.ben'sis N.L. fem. adj. onubensis, originating from Onuba, the Roman name of Huelva, the province where the type strain was isolated).

Cells are Gram-positive, aerobic, motile, rod-shaped, 0.6-0.9 µm wide and 2.8-3.8 µm long, occurring singly, in pairs or in chains. Ellipsoidal endospores are produced within swollen sporangia, centrally or sub-terminally. Colonies grown for two days on TSA at 30°C are 1-3 mm in diameter, convex, yellowish, translucent, with glossy appearance and irregular margins. Catalase-positive and oxidase-negative. Growth occurs between 10 and 47°C, optimum at 37°C. Grows at 0-8 % (w/v) NaCl with an optimum at 1 % (w/v) NaCl. Tolerates a

range of pH between 6 and 9.5. Aesculin and Tween 20 are hydrolysed. Gelatin, Tween 40 and 80 are not hydrolysed. Nitrate is reduced to nitrite. Indole is not produced from tryptophan and glucose fermentation does not occur. Positive for α-chymotrypsin, naphthol-AS-BI-phosphohydrolase and α-glucosidase activities. Weakly positive for esterase (C4), esterase lipase (C8), leucine arylamidase, and trypsin activities. Negative for arginine dihydrolase, urease, acid phosphatase, lipase (C14), valine arylamidase, cysteine arylamidase, alkaline phosphatase, α- and β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities. Assimilates glucose, N-acetylglucosamine, maltose and malate, but not arabinose, mannose, mannitol, potassium gluconate, capric acid, adipic acid, phenylacetic acid and trisodium citrate. Acid is produced from aesculin, D-fructose, D-glucose, D-maltose, N-acetylglucosamine, D-saccharose, D-tagatose and D-trehalose. Acid production is weak from D-galactose, D-lactose, D-turanose and D-xylose. Acid production is variable from L-arabitol, L-fucose, D-melezitose and starch. Acid is not produced from D-adonitol, amygdalin, D- and L-arabinose, D-arabitol, arbutin, D-cellobiose, dulcitol, erythritol, D-fucose, gentibiose, glycerol, glycogen, inositol, inulin, D-lyxose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-β-D-xylopyranoside, D-mannitol, D-mannose, D-melibiose, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, D-raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, xylitol and L-xylose. The major cellular polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and two phospholipids. Predominant fatty acids are *iso*-$C_{15:0}$ and *anteiso*-$C_{15:0}$. Susceptibility to amoxicillin/clavulanic acid, ampicillin, ciprofloxacin, gentamicin,

norfloxacin, streptomycin, tobramycin, penicillin, rifampicin, amikacin, azithromycin, cefuroxime, clindamycin, erythromycin, kanamycin, nalidixic acid, novobiocin, oxytetracycline, tetracycline and vancomycin. Resistant to fosfomycin+glucose-6-phosphate and lincomycin. MK-7 is the dominant isoprenoid quinone. The G+C content of the type strain is 38 mol %.

The type strain, 0911MAR22V3^T (=LMG 27963^T =CECT 8479^T), was isolated from an air sample collected in Gruta de las Maravillas (Aracena, Spain); a reference strain, 0911TES10J4 (CECT 8478) was isolated from an air sample collected in Cueva del Tesoro (Rincon de la Victoria, Spain).

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Fig. 1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strains 0911MAR22V3^T, 0911TES10J4 and other species of the genus *Bacillus*. Bootstrap values (>50 %) are expressed as percentages of 1000 replicates. Closed circles show the nodes that were also recovered by the neighbor-joining and maximum-parsimony algorithms. The 16S rRNA gene sequence of *Paenibacillus brasiliensis* DSM 14914^T was used as the outgroup. Bar, 0.01 substitutions per nucleotide position.

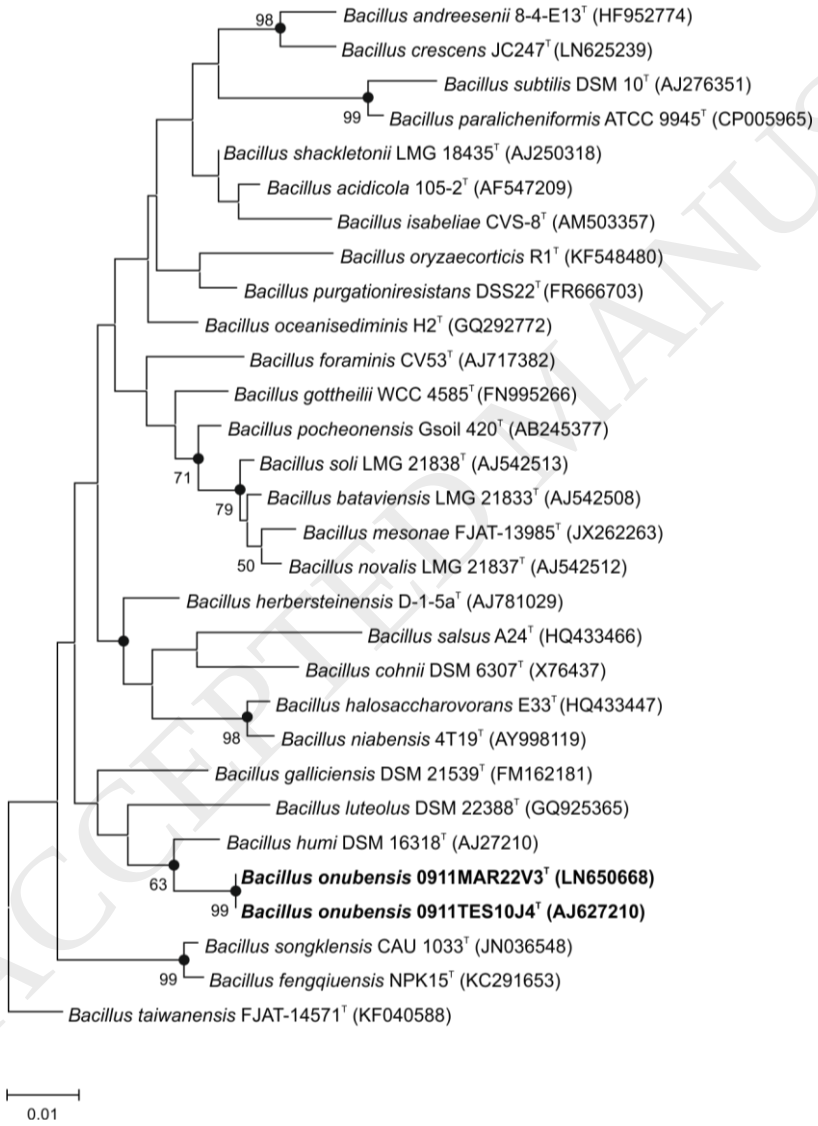

Fig. 2. Field emission scanning electron images of strain 0911MAR22V3^{T} cells. A) General view showing cell morphology. B) Detailed view of a swollen sporangia with sub-terminal endospore indicated by an arrow. Scale bars, 1 µm.

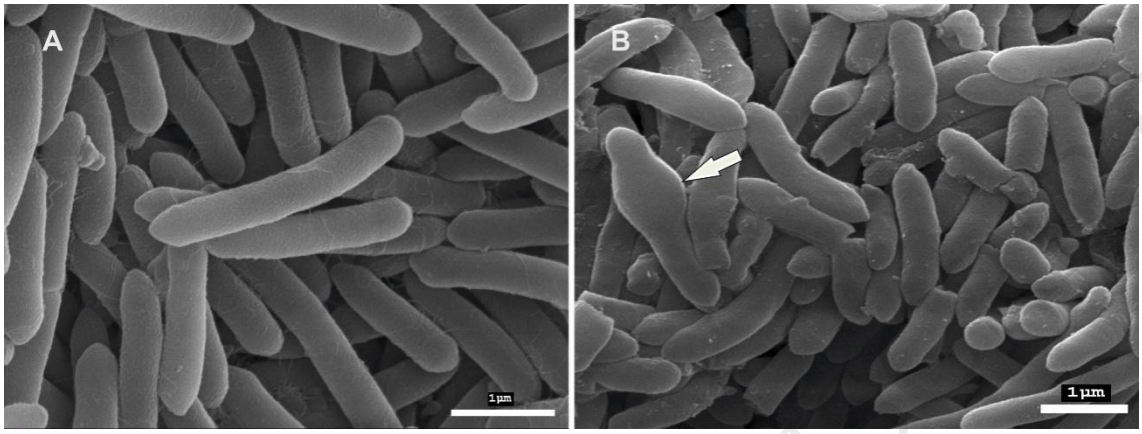

Table 1. Differential characteristics of strains 0911MAR22V3^T, 0911TES10J4 and related species.

Characteristic	1	2	3	4	5
Growth conditions					
Temperature					
8°C	-	-	-	+	-
37°C	+	+	+	-	+
40°C	+	+	+	-	-
47°C	(+)	+	-	-	-
pH					
pH 6	(+)	+	+	-	-
NaCl concentration					
8 % (w/v) NaCl	(+)	(+)	-	-	(+)
Anaerobic growth	-	(+)	(+)	(+)	-
Oxidase	-	-	+	+	-
Hydrolysis of:					
Tween 20	+	+	-	+	+
Tween 40	-	-	+	+	+
Tween 80	-	-	+	+	+
Enzymatic activity:					
Alkaline phosphatase	-	-	+	(+)	(+)
Leucine arylamidase	(+)	+	+	-	+
Cystine arylamidase	-	(+)	(+)	-	-
Trypsin	(+)	-	-	-	-
Acid phosphatase	-	-	+	-	(+)
β-Glucuronidase	-	-	+	-	-
α-Glucosidase	+	+	-	+	+
β-Glucosidase	-	-	-	+	-
Acid production from:					
D-Adonitol	-	+	-	(+)	-
Amygdalin	-	+	±	+	-
D-Arabinose	-	+	(+)	+	-
L-Arabinose	-	+	(+)	+	-
L-Arabitol	±	+	-	-	-
Arbutin	-	-	-	(+)	-
D-Cellobiose	-	+	(+)	+	-
Dulcitol	-	-	-	(+)	-
Erythritol	-	-	-	(+)	-
L-Fucose	±	(+)	(+)	-	-
D-Galactose	(+)	(+)	(+)	+	-
Gentibiose	-	-	(+)	+	-
D-Glucose	+	+	(+)	+	-
Glycerol	-	(+)	-	+	(+)
Glycogen	-	-	+	(+)	(+)
Inositol	-	(+)	-	(+)	-
Inulin	-	+	(+)	-	-
D-Lactose	(+)	+	(+)	+	-
D-Lyxose	-	-	(+)	-	-
D-Melezitose	±	(+)	-	(+)	-

Table 1. Continuation.

Characteristic	1	2	3	4	5
Acid production from:					
Methyl-α-D-glucopyranoside	-	(+)	-	+	-
Methyl-β-D-xylopyranoside	-	-	-	+	-
D-Mannitol	-	-	-	+	-
D-Mannose	-	-	(+)	+	-
D-Melibiose	-	(+)	(+)	+	-
N-Acetylglucosamine	+	+	(+)	+	-
Potassium 5-ketogluconate	-	(+)	-	-	-
D-Raffinose	-	+	-	+	-
D-Ribose	-	±	(+)	(+)	-
Salicin	-	(+)	(+)	+	-
D-Sorbitol	-	-	-	(+)	-
L-Sorbose	-	-	-	(+)	-
Starch	±	(+)	-	+	(+)
D-Tagatose	+	-	-	-	-
D-Turanose	(+)	-	(+)	(+)	-
Xylitol	-	(+)	-	-	-
L-Xylose	-	-	-	(+)	-
Assimilation of:					
Glucose	+	+	-	-	-
Arabinose	-	+	+	-	-
Mannose	-	-	+	-	-
N-Acetylglucosamine	+	+	+	-	-
Maltose	+	+	+	-	-
Potassium gluconate	-	-	(+)	-	-
Malate	+	+	+	-	-
Trisodium citrate	-	-	+	-	-
Glucose fermentation	-	-	-	-	+
Nitrate reduction	+	+	+	-	-
Antibiotic susceptibility:					
Fosfomycin+Glucose-6-Phosphate	-	+	-	-	+
Lincomycin 2 µg	-	-	+	-	-
G+C content (mol %)	38	38.1	37.5 [†]	48.1[#]	36.9[‡]

Strains: 1, 0911MAR22V3^T; 2, 0911TES10J4; 3, *Bacillus humi* DSM 16318^T; 4, *Bacillus galliciensis* DSM 21539^T, 5, *Bacillus luteolus* DSM 22388^T. +, Positive; (+), weak positive; ±, variable; -, negative. Antibiotic susceptibility: +, antibiotic sensibility; -, antibiotic resistance. Data were obtained in this study unless indicated. Data taken from: [†], Heyrman *et al*. [11]; [#], Balcázar *et al*. [3]; [‡], Shi *et al*. [28].

Table 2. Results of ANI calculations (%) and TETRA correlations of strains 0911MAR22V3^T, 0911TES10J4, *Bacillus humi* and the type species of the genus, *Bacillus subtilis.*

	0911MAR22V3^T	**0911TES10J4**	***Bacillus humi* DSM 16318^T**	***Bacillus subtilis* strain 168**
ANIb				
911MAR22V3^T	100	93.59	81.64	67.05
0911TES10J4	93.81	100	81.67	67.29
B. humi	81.72	81.68	100	67.19
B. sutilis	67.92	67.77	67.70	100
ANIm				
911MAR22V3^T	100	94.62	84.96	85.33
0911TES10J4	94.62	100	84.90	85.76
B. humi	84.96	84.90	100	85.05
B. sutilis	85.33	85.78	85.02	100
OrthoANI				
911MAR22V3^T	100			
0911TES10J4	94.28	100		
B. humi	82.26	82.20	100	
B. sutilis	68.41	68.71	68.45	100
TETRA				
911MAR22V3^T		0.99864	0.98234	0.74916
0911TES10J4	0.99864		0.98134	0.74143
B. humi	0.98234	0.98134		0.76933
B. sutilis	0.74916	0.74143	0.76933	