

Paenibacillus aquistagni sp. nov., isolated from an artificial lake accumulating industrial wastewater

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Abstract Strain 11^T was isolated from water of an artificial lake accumulating industrial wastewater on the outskirts of Celje, Slovenia. Phenotypic characterisation showed strain 11^T to be a Gram-stain positive, spore forming bacterium. The 16S rRNA gene sequence identified strain 11^T as a member of the genus *Paenibacillus*, closely related to *Paenibacillus alvei* (96.2%). Genomic similarity with *P. alvei* 29^T was 73.1% (gANI), 70.2% (ANIb), 86.7% (ANIm) and 21.7 ± 2.3% (GGDC). The DNA G+C content of strain 11^T was determined to be 47.5%. The predominant menaquinone of strain 11^T was identified as MK-

7 and the major fatty acid as anteiso-C_{15:0}. The peptidoglycan was found to contain *meso*-diaminopimelic acid. In contrast to its close relatives *P. alvei* DSM 29^T, *Paenibacillus apiarius* DSM 5581^T and *Paenibacillus profundus* NRIC 0885^T, strain 11^T was found to be able to ferment D-fructose, D-mannose and D-xylose. A draft genome of strain 11^T contains a cluster of genes associated with type IV pilin synthesis usually found in clostridia, and only sporadically in other Gram-positive bacteria. Genotypic, chemotaxonomic, physiological and biochemical characteristics of strain 11^T presented in this study support the creation of a novel species within the genus *Paenibacillus*, for which the name *Paenibacillus aquistagni* sp. nov. is proposed, with strain 11^T (=ZIM B1027^T =LMG 29561^T =CCM 8679^T) as the type strain.

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Introduction

The genus *Paenibacillus* was proposed by Ash et al. (1993) for the members of *Bacillus* group 3; later, its description was emended by Shida et al. (1997). Presently, *Paenibacillus* spp. are classified into over 200 validly named species (<http://www.bacterio.net/paenibacillus.html>) with *Paenibacillus polymyxa* as

the type species (Ash et al. 1993). Most *Paenibacillus* species consist of strains that are motile, Gram-stain positive (some are Gram-stain variable, such as *Paenibacillus taiwanensis* and *Paenibacillus assamensis*), spore forming, facultatively anaerobic or strictly aerobic. Most species are catalase positive and show optimal growth at 28–40 °C and pH 7.0, with some species being alkaliphilic. The major fatty acid of *Paenibacillus* spp. is anteiso-C_{15:0}. The DNA G+C content of *Paenibacillus* spp. ranges from 40 to 59 mol% (Priest 2015).

Paenibacillus species have been isolated from diverse habitats, such as paperboard, deep-sea surface sediment, honeybee larva, warm springs, farmland soil, tree bark, blood samples, sputum, human urine, rhizosphere soil and necrotic wounds (Nakamura 1996; Roux and Raoult 2004; Saha et al. 2005; Lee et al. 2007; Roux et al. 2008; Kim et al. 2010; Romanenko et al. 2013; Han et al. 2015; Sitdhipol et al. 2016). During an assessment of the cultivable microbial diversity from water of an artificial lake located on the outskirts of Celje (Slovenia) which is accumulating liquid industrial waste, strain 11^T was isolated. In a phylogenetic tree based on the 16S rRNA gene sequence, strain 11^T formed a separate branch within the genus *Paenibacillus*, suggesting it could represent a novel species. To clarify its taxonomic status, further genotypic and phenotypic analyses were performed. The genome of strain 11^T was sequenced under the umbrella of the Genomic Encyclopedia of Type Strains, Phase III: the genomes of soil and plant-associated and newly described type strains (Whitman et al. 2015).

Materials and methods

Strains and growth conditions

A lake situated on the outskirts of Celje (Slovenia) was sampled in September 2013. The water sample had a pH of 7.3. Strain 11^T was isolated by plating the sampled water on nutrient agar (NA, Sigma-Aldrich) and incubating the plates at 25 °C in an aerobic atmosphere for 4 days.

The reference type strains *Paenibacillus alvei* DSM 29^T and *Paenibacillus apiarius* DSM 5581^T were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany) and the

type strain *Paenibacillus profundus* NRIC 0885^T from the Nodai Culture Collection (NRIC, Japan). All reference strains were cultured on NA medium for comparative analyses.

Morphological, physiological and biochemical analysis

Colonial morphology and size were described and measured visually after cultivation on NA medium. Gram-stain reaction was determined by using a Gram-staining kit (Sigma-Aldrich, St. Louis, MO, USA), following the instructions of the manufacturer. Cytochrome *c* oxidase activity was determined by oxidation of 1% (w/v) *N,N,N',N'*-tetramethyl-1,4-phenylenediamin dihydrochloride and catalase activity by observing air bubble production using 3% (v/v) H₂O₂ as described by Gerhardt et al. (1994). Oxygen demand for growth was determined by incubating inoculated NA medium for 7 days in a jar with anaerobic atmosphere generation bags (Sigma-Aldrich). Resistance against different concentrations of NaCl and the pH range for growth were analysed in nutrient broth. The growth was detected by measuring an increase in turbidity. The temperature range for growth was analysed on NA medium by incubating the plates for 10 days at different temperatures. Motility was determined as described by Alexander and Strete (2001) in a tube with semisolid NA medium containing 0.5% agar by incubating the tube for 4 days at 30 °C. Gliding motility was analysed on the same type of medium in a Petri dish by inoculating the surface of the medium with a spot of strain 11^T culture. The presence of endospores was determined according to the method of Schaeffer-Fulton as described by Gerhardt et al. (1994).

Other biochemical characteristics were determined using the API 50CH, API ZYM and API 20E microtest systems (bioMérieux, Marcy l'Etoile, France) at 30 °C, following the manufacturer's instructions.

Phylogenetic analysis

The 16S rRNA gene sequence of strain 11^T (1478 nt) was determined as described previously (Trček et al. 2000) with the following modifications: DNA was isolated with a GeneJETTM Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) following the protocol for Gram-positive

bacteria. The 16S rRNA gene sequence was amplified with primers fD1 (5'-AGAGTTTGATCMTGGCT-CAG-3') and rH1542 (5'-AAGGAGGTGATC-CAGCCGCA-3'). The nucleotide sequences of both strands were determined by the primer walking method using the primers described by Boesch et al. (1998). The 16S rRNA gene sequence was compared to all type strains representing the genus *Paenibacillus*. The sequences were aligned with the Clustal X 1.8 program (Thompson et al. 1997) using the default settings of the multiple alignment tool and then subjected to the phylogenetic analyses by applying the neighbour-joining method in the DNADIST program from the PHYLIP package (Felsenstein 2002). The tree was drawn with the Treeview program (Page 2000). The robustness of the phylogenetic tree topology was evaluated by bootstrap analysis with 1000 replications using the SEQBOOT and CONSENCE programs from the PHYLIP package. Bootstrap values >60% were considered to be significant.

Sequencing of the genome

Genomic DNA was extracted with a NucleoSpin Tissue kit (Macherey–Nagel) using the manufacturer's protocol. A draft genome sequence of strain 11^T was generated at the DOE Joint Genome Institute (JGI). An Illumina 300 bp insert standard shotgun library was constructed and sequenced using the Illumina HiSeq 2000-1 TB platform. The reads were filtered using BBDuk (Bushnell 2014), which removes known Illumina artifacts and spike-in PhiX control. Reads with more than one “N” or with quality scores (before trimming) averaging less than 8 or reads shorter than 51 bp (after trimming) were discarded. Assembly was performed with SPAdes (version 3.6.2) (Bankevich et al. 2012). Assembly contigs were discarded if the length was <1 kbp.

Genome annotation

The JGI Microbial Genome Annotation Pipeline (MGAP v.4) (Huntemann et al. 2015; Chen et al. 2016) was used for annotating the draft genome, followed by a round of manual curation using the JGI GenePRIMP pipeline (Pati et al. 2010). The annotated genome is available from the Integrated Microbial Genomes (IMG) system (Chen et al. 2017).

Chemotaxonomic characterisation

The whole cell fatty acids of strain 11^T, *P. alvei* DSM 29^T, *P. apiarius* DSM 5581^T and *P. profundus* NRIC 0885^T, all grown for 24 h at 28 °C under aerobic conditions on trypticase soy agar (TSA, Difco) medium, were determined by GC using an Agilent Technologies 6890 N gas chromatograph (Santa Clara, CA, USA). The peak naming table MIDI TSBA 5.0 was used.

The isomer type of the diamino acid in the cell wall of strain 11^T was analysed by thin-layer chromatography as described previously (Schumann 2011).

Extraction of quinones was carried out according to the two stage method described by Tindall (1990a, b). Quinones were separated by thin layer chromatography on silica gel (Macherey–Nagel Art. No. 805 023), using hexane:tert-butylmethylether (9:1 v/v) as solvent. UV absorbing bands corresponding to menaquinones were further analysed using a LDC Analytical (Thermo Separation Products) HPLC fitted with a reverse phase column (Macherey–Nagel, 2 mm × 125 mm, 3 µm, RP18) using methanol:heptane 9:1 (v/v) as the eluant. Menaquinones were detected at 269 nm.

Results and discussion

Phenotypic characteristics

The cells of strain 11^T were observed to be Gram-stain positive, spore-forming and facultatively anaerobic rods of 0.4–0.6 µm in width and 1.5–2.5 µm in length. The colonies of strain 11^T grown on NA medium for 48 h at 30 °C were observed to be white and of irregular shape. Optimal growth of strain 11^T was observed at 30 °C and pH 7. Growth was observed from 10 to 46 °C, pH 5.5 to 13 and 0 to 1.5% NaCl. Strain 11^T was found to be oxidase and catalase positive. In API ZYM tests, strain 11^T was found to be positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase and α-glucosidase. Lipase (C14), valine arylamidase, acid phosphatase, β-glucosidase cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase tests are negative. In the API 50CH system, strain 11^T ferments the following carbon

sources: glycerol, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, methyl- α D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-raffinose, starch (amidon), gentiobiose, and D-turanose. Methyl- α D-mannopyranoside, L-fucose and glycogen are weakly fermented. Erythritol, D-arabinose, L-arabinose, L-xylose, D-adonitol, methyl- β -D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, inulin, D-melezitose, xylitol, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate are not used as carbon sources. In the API 20E system, nitrate is not reduced to nitrite, gelatin is not liquefied, and H₂S and indole are not produced. β -galactosidase, arginine dihydrolase, lysine dihydrolase, lysine decarboxylase and tryptophan deaminase activities were not detected. The main phenotypic characteristics differentiating strain 11^T from its close relatives *P. alvei* DSM 29^T, *P. apiarius* DSM 5581^T and *P. profundus* SI 79^T are fermentation of D-fructose, D-mannose and D-xylose, no liquefaction of gelatin, positive α -glucosidase activity, and negative α -chymotrypsin and acid phosphatase activities (Table 1). These characteristics are noted to be similar to those of strain “*Paenibacillus tyraminigenes*” H3209 (Table 1), which has been proposed to represent a taxon whose name has not been validated (Mah et al. 2008).

Chemotaxonomic characteristics

The major fatty acids of strain 11^T were identified as anteiso-C_{15:0} (53.6%), iso-C_{15:0} (11.7%), iso-C_{16:0} (8.4%), iso-C_{17:0} (5.8%), anteiso-C_{17:0} (5.4%) and C_{16:0} (5.5%). The overall fatty acid composition of the strain 11^T was similar to those of closely related *Paenibacillus* species (Table 2). The quinone analysis showed that strain 11^T contains menaquinone MK-7 as the predominant quinone (92%), which is in accordance with findings reported for other *Paenibacillus* species (Ludwig et al. 2009). The diamino acid of the cell wall peptidoglycan was determined to be meso-diaminopimelic acid.

Molecular analyses

Phylogenetic analysis using a nearly complete 16S rRNA gene sequence of strain 11^T (1478 nt) revealed that strain

Table 1 Phenotypic characteristics of *Paenibacillus aquis-tagni* sp. nov. in comparison to type strains of closely related species (1 *P. aquis-tagni* sp. nov.; 2 *P. alvei* DSM 29^T; 3 *P. apiarius* DSM 5581^T; 4 *P. profundus* NRIC 0885^T)

Characteristic	1	2	3	4	5
Growth at					
45 °C	+	+	–	–	+
10 °C	+	–	–	+	–
pH = 4.6	–	–	+	–	na
pH = 5.6	+	+	–	+	na
2% NaCl	–	–	+	+	+
5% NaCl	–	–	+	+	+
Acid production from					
D-Fructose	+	–	–	–	+
L-Fucose	w	–	–	+	na
D-Mannose	+	–	w	–	na
D-Xylose	+	–	–	–	+
Methyl- α D-mannopyranoside	w	–	–	w	na
Gelatin liquefaction	–	+	+	+	–
H ₂ S production	–	–	–	+	na
Voges-Proskauer reaction	w	+	+	w	–
Enzyme activities					
β -Galactosidase	+	–	+	+	na
Arginine dihydrolase	–	–	+	–	na
α -Chymotrypsin	–	+	+	+	na
Acid phosphatase	–	+	+	+	na
α -Glucosidase	+	–	–	–	na

All data in these columns are from this study. Column 5, data available for the closely related but unavailable strain “*P. tyraminigenes*” H3209 (Mah et al. 2008)

+ positive, – negative; w weakly positive; na not available

11^T belongs to the genus *Paenibacillus* and is closely related to strain “*P. tyraminigenes*” H3209, *P. alvei* DSM 29^T, *P. apiarius* DSM 5581^T, *P. profundus* SI 79^T and *P. taiwanensis* BCRC 17411^T with 99.8, 96.2, 94.9, 94.8 and 94.7% sequence similarity, respectively. Nucleotide identity between the 16S rRNA gene sequence of strain 11^T and other species within the genus was found to be below 94.4%. The closely related strain “*P. tyraminigenes*” H3209 has been deposited in the Korean Collection for Type Cultures as a patent strain (KCTC 10694BP) and described in Mah et al. (2008) as the type strain of a novel *Paenibacillus* species. However, since the species name “*P. tyraminigenes*” H3209 has not been validated and, moreover, the strain is not available (or deposited in two culture collections), its name has no standing in the nomenclature.

Table 2 Fatty acid composition (%) of *Paenibacillus aquis-tagni* sp. nov. and the type strains of closely related species (1 *P. aquis-tagni* sp. nov.; 2 *P. alvei* DSM 29^T; 3 *P. apiarius* DSM 5581^T; 4 *P. profundus* NRIC 0885^T)

Fatty acid	1	2	3	4
C _{10:0}	0.3	0.7	0.3	0.3
anteiso-C _{11:0}	0.4	nd	0.4	0.6
C _{12:0}	0.6	2.3	1.0	0.6
iso-C _{13:0}	0.7	nd	nd	nd
anteiso-C _{13:0}	1.0	nd	nd	nd
C _{13:0}	0.5	nd	nd	nd
iso-C _{14:0}	4.9	1.8	0.9	1.5
C _{14:0}	1.2	2.7	1.6	1.5
iso-C _{13:0} 3OH	nd	1.0	nd	nd
C _{13:0} 2OH	nd	2.4	nd	nd
iso-C _{15:0}	11.7	6.2	13.2	11.9
anteiso-C _{15:0}	53.6	51.9	48.8	51.8
C _{15:0}	nd	nd	nd	nd
C _{16:1} ω7c alcohol	nd	1.2	0.8	1.7
iso-C _{16:0}	8.4	7.0	5.0	7.8
C _{16:1} ω11c	nd	4.1	3.8	4.1
C _{16:0}	5.5	9.2	7.9	4.4
iso-C _{17:1} ω10c	nd	nd	1.7	1.4
iso-C _{17:0}	5.8	2.8	4.8	4.3
anteiso-C _{17:0}	5.4	6.0	7.0	6.4
C _{18:0}	nd	nd	1.6	nd

All data are from this study

nd not detected

In a phylogenetic NJ tree (Fig. 1), strain 11^T forms a distinct branch well separated from its close relative *P. alvei* DSM 29^T. Since the 16S rRNA gene sequence identity of strain 11^T to the type strains of the closely related species was found to be significantly below the threshold of 98.7–99% (Stackebrandt and Ebers 2006) at which DNA–DNA hybridization (DDH) experiments are mandatory, wet-lab hybridization analyses were not performed. The DNA G+C content of strain 11^T is 47.5% (estimated from a draft genome sequence determined in the frame of this study, see below). This value falls within the expected range of 40–59 mol% reported for the members of the genus *Paenibacillus* (Priest 2015).

Sequence analysis of the genome

Sequencing of the genome of strain 11^T produced a final draft assembly which contained 18 contigs in 18

scaffolds, totalling 5.386 Mbp in size based on 1,183.2 Mbp of Illumina data with a mapped coverage of 220.1X. Annotation with the JGI Microbial Genome Annotation Pipeline (MGAP v.4) (Hunte-mann et al. 2015) identified 4863 (97.7%) protein coding sequences (CDS), of which a function has been predicted for 3804 (76.4%), along with 17 rRNA genes (seven 5S rRNA, seven 16S rRNA and three 23S rRNA) and 77 tRNA genes.

Comparison of the draft genome sequence of strain 11^T to that of strains of the closely related species *P. alvei* yielded ANI values of around 73% (gANI) (Varghese et al. 2015), 70% (ANIb) and 86% (ANIm) (Table 3). These values are clearly below the species-delineating threshold of 96% (Richter and Rosselló-Móra 2009; Varghese et al. 2015), meaning that strain 11^T does not belong to the species *P. alvei*.

The genome to genome distance calculator (GGDC) (Auch et al. 2010; Meier-Kolthoff et al. 2013) was used to calculate the genomic distance between strain 11^T and strains of closely related species. The GGDC values for *P. alvei* strains DSM 29^T, A6-6i-x, E194 and TS-15, when compared to strain 11^T, were 21.7 ± 2.3, 23.2 ± 2.3, 22.8 ± 2.3 and 23.0 ± 2.3%, respectively. The GGDC comparison for some other closely related species, *P. assamensis* DSM 18201^T, *P. taiwanensis* DSM 18679^T, *Paenibacillus popilliae* ATCC 14706^T and *Paenibacillus lentimorbus* NLLR B-30488, yielded the following values: 21.7 ± 2.3, 19.5 ± 2.3, 19.4 ± 2.3 and 19.0 ± 2.4%, respectively. All these values are well below the threshold value of 70%, which confirms that strain 11^T does not belong to *P. alvei* or any of these species.

Analysis of the draft genome of strain 11^T has shown that it contains a cluster of genes related to those encoding a type four pilus system. The genes in the cluster are organised similarly to the *pil* operons in *Clostridium* and *Bacillus* species (Imam et al. 2011). Initially considered to be exclusive to Gram-negative bacteria, they were later found in *Streptococcus sanguinis* and *Clostridium difficile* (Fives-Taylor and Thompson 1985; Borriello et al. 1990). Genomic information available since then has shown that a vast majority of Gram-negative and almost all clostridial species contain type four pilin (TFP) associated genes. On the other hand, TFP genes are present only sporadically in other Gram-positive species (Melville and Craig 2013). Strain 11^T contains all the components for the synthesis of pili, including genes for type IV pilus

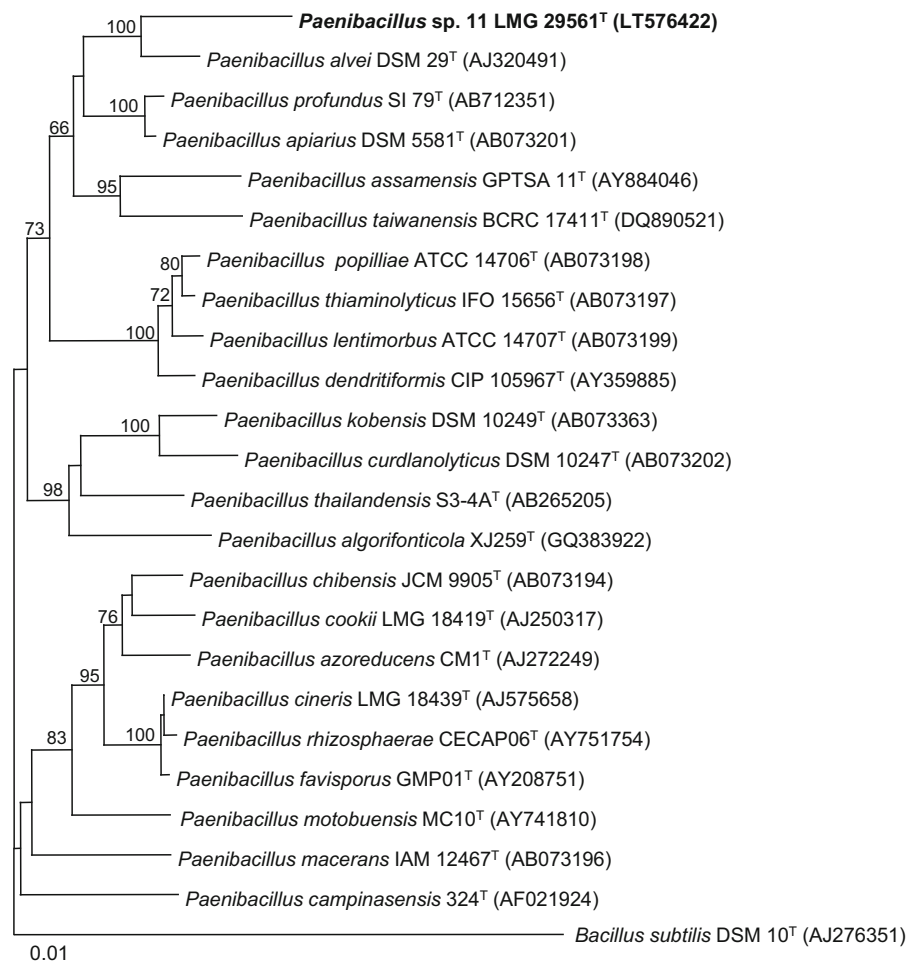


Fig. 1 Phylogenetic tree reflecting the relationships between *Paenibacillus aquistagni* sp. nov. and the type strains of closely related species of the genus *Paenibacillus* based on 16S rRNA gene sequences. The tree was constructed by the neighbour-joining method. *Bacillus subtilis* is included as an outgroup.

Bootstrap values based on 1000 replicates are indicated at branch points, only numbers greater than 60% are shown. The bar indicates 1% sequence differences. The accession numbers are in parenthesis

assembly protein PilB (SMG56614), twitching motility protein PilT (WP_085497616), type IV pilus assembly protein PilC (WP_085497614), type IV pilin PilA (SMG56607), leader peptidase (prepilin peptidase)/N-methyltransferase (PilD) (WP_085497612), type IV pilus assembly protein PilM (WP_085497609), type IV pilus assembly protein PilN (WP_085497608) and a hypothetical protein (WP_085497605) with a 31% homology to a type IV pilus assembly protein PilO found in *Bacillus soli* (WP_066063795). The open access PilFind program (Imam et al. 2011) also identified PilA (SMG56607) as a TFP. Out of more than 200 *Paenibacillus* strains sequenced to this date, approximately 40 (including strain 11^T) code for a pilus

forming protein PilA. However, only seven sequenced *Paenibacillus* genomes (including that of strain 11^T) out of more than 200, code for the inner membrane accessory proteins PilM, PilN and PilO, which are known to form a complex indispensable for pilus assembly in *Pseudomonas aeruginosa* (Ayers et al. 2009). This indicates that TFP might be a rare trait in *Paenibacillus* species. Indeed, the genomes of none of the close relatives of strain 11^T contain this cluster. TFP are large and varied protein filaments involved in a diverse array of cellular functions, including gliding motility, conjugation, adherence, DNA uptake, and biofilm formation, all adding to pathogenic properties of bacteria. While they are frequent in Gram-negative

Table 3 Results of ANI calculations (%) using MiSI (gANI) and JSpecies (ANiB and ANIm) software

	1	2	3	4	5	6	7	8	9
gANI									
1. <i>P. aquistagni</i> sp. nov. 11 ^T	–	73.1	73.3	73.4	73.3	72.7	73.1	72.5	67.5
2. <i>P. alvei</i> DSM 29 ^T	73.1	–	85.2	85.3	85.3	72.6	73.0	74.0	67.4
3. <i>P. alvei</i> A6-6i-x	73.2	85.3	–	95.9	95.9	73.0	73.3	74.0	67.3
4. <i>P. alvei</i> E194	73.3	85.3	95.9	–	98.1	72.9	73.6	74.3	67.3
5. <i>P. alvei</i> TS-15	73.2	85.3	95.9	98.1	–	73.0	73.4	74.0	66.9
6. <i>P. assamensis</i> DSM 18201 ^T	72.7	72.6	72.7	72.9	73.0	–	72.8	72.0	67.5
7. <i>P. taiwanensis</i> DSM 18679 ^T	73.1	73.0	73.3	73.6	73.5	72.8	–	72.3	67.0
8. <i>P. popilliae</i> ATCC 14706 ^T	72.4	74.0	74.0	74.3	74.0	72.0	72.3	–	67.6
9. <i>P. lentimorbus</i> NRRL B-30488	67.7	67.9	67.4	67.3	67.2	67.5	67.2	67.6	–
ANiB									
1. <i>P. aquistagni</i> sp. nov. 11 ^T	–	70.2	70.3	70.4	70.4	69.8	70.2	69.6	64.3
2. <i>P. alvei</i> DSM 29 ^T	70.2	–	83.9	84.2	84.0	69.4	69.9	71.5	65.1
3. <i>P. alvei</i> A6-6i-x	70.0	83.9	–	95.0	95.0	69.3	70.2	71.2	64.2
4. <i>P. alvei</i> E194	69.9	83.9	94.8	–	97.6	69.5	70.2	71.3	64.1
5. <i>P. alvei</i> TS-15	69.9	83.7	94.8	97.6	–	69.3	69.9	70.8	63.9
6. <i>P. assamensis</i> DSM 18201 ^T	69.6	69.8	69.8	69.8	69.8	–	69.7	68.9	64.2
7. <i>P. taiwanensis</i> DSM 18679 ^T	70.3	70.1	70.3	70.5	70.5	69.8	–	69.5	64.1
8. <i>P. popilliae</i> ATCC 14706 ^T	69.9	71.7	71.9	72.1	71.8	68.9	69.6	–	65.1
9. <i>P. lentimorbus</i> NRRL B-30488	64.3	64.5	64.4	64.4	64.3	64.3	64.0	65.0	–
ANIm									
1. <i>P. aquistagni</i> sp. nov. 11 ^T	–	86.7	86.3	86.7	86.6	86.4	84.5	85.1	85.4
2. <i>P. alvei</i> DSM 29 ^T	86.7	–	87.5	87.7	87.5	86.1	84.7	84.3	86.7
3. <i>P. alvei</i> A6-6i-x	86.3	87.5	–	95.6	95.6	86.2	85.8	84.1	87.1
4. <i>P. alvei</i> E194	86.7	87.7	95.6	–	97.9	86.0	85.6	85.2	87.9
5. <i>P. alvei</i> TS-15	86.6	87.5	95.6	97.9	–	85.9	85.5	84.3	87.9
6. <i>P. assamensis</i> DSM 18201 ^T	86.4	86.1	86.1	86.0	85.9	–	84.4	84.4	91.3
7. <i>P. taiwanensis</i> DSM 18679 ^T	84.5	84.7	85.8	85.5	85.5	84.4	–	84.3	86.3
8. <i>P. popilliae</i> ATCC 14706 ^T	85.0	84.5	84.1	85.2	84.3	84.4	84.5	–	86.2
9. <i>P. lentimorbus</i> NRRL B-30488	85.4	86.7	87.1	87.9	87.9	90.1	86.42	86.2	–

Comparison between 1 *P. aquistagni* sp. nov. 11^T (FXAZ01000000), 2 *P. alvei* DSM 29^T (NZ_AMBZ00000000), 3 *P. alvei* A6-6i-x (ATMS00000000), 4 *P. alvei* E194 (NZ_JMDX00000000), 5 *P. alvei* TS-15 (ATMT00000000), 6 *P. assamensis* DSM 18201^T (NZ_AULU01000000), 7 *P. taiwanensis* DSM 18679^T (NZ_AULE00000000), 8 *P. popilliae* ATCC 14706^T (BALG00000000), 9 *P. lentimorbus* NRRL B-30488 (ANAT00000000)

bacteria, their presence and role in Gram-positive bacteria has only recently been discovered (Craig et al. 2004; Melville and Craig 2013). The genome sequence of strain 11^T will therefore be valuable in expanding our knowledge of TFP systems in Gram-positive bacteria.

The results of this polyphasic study demonstrate that strain 11^T belongs to the genus *Paenibacillus*. Differences in phenotypic, as well as phylogenetic and genomic characteristics distinguish strain 11^T from previously described validly named *Paenibacillus* species, and thus it is considered that strain 11^T

represents a novel *Paenibacillus* species for which the name *Paenibacillus aquistagni* sp. nov. is proposed. The Digital Protologue database (Rosselló-Móra et al. 2017) TaxoNumber for strain 11^T is TA00102.

Description of *Paenibacillus aquistagni* sp. nov.

Paenibacillus aquistagni (a.qui'stag.ni. L. n. *aqua* water; L. gen. n. *stagni* of the lake; N.L. gen. n. *aquistagni* of the water of the lake).

Cells are Gram-stain positive, spore forming, facultatively anaerobic, oxidase positive, catalase positive, motile rods (0.4–0.6 × 1.5–2.5 µm). Forms irregularly shaped white colonies of 1.2–2.0 mm and 2.5–3.0 mm in diameter on NA medium after 2 and 5 days of cultivation, respectively. Growth occurs at 10–46 °C and pH 5.5–13, with optimal growth at 30 °C and pH 7. Tolerates a maximum of 1.5% NaCl. Nitrate is not reduced to nitrite. Gelatin is not liquefied. H₂S and indole are not produced. Urease activity is not detected. The predominant fatty acids are anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{16:0}, iso-C_{17:0}, anteiso-C_{17:0} and C_{16:0}. The major menaquinone is MK-7. Contains meso-diaminopimelic acid as the diagnostic diamino acid of the peptidoglycan. The DNA G+C content of the type strain is 47.5% (estimated from a draft genome sequence).

The type strain, 11^T (=ZIM B1027^T=LMG 29561^T=CCM 8679^T), was isolated from water of an artificial lake. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 11^T is LT576422. The draft genome sequence of strain 11^T has been deposited at ENA under the accession number FXAZ01000000.

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Conflict of interest The authors declare that they have no conflict of interest.

References

- Alexander SK, Strete D (2001) Microbiology: a photographic atlas for the laboratory. Benjamin Cummings, San Francisco
- Ash C, Priest FG, Collins MD (1993) Molecular identification of rRNA group bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. Proposal for the creation of a new genus *Paenibacillus*. Antonie Van Leeuwenhoek 64:253–260
- Auch AF, von Jan M, Klenk HP, Göker M (2010) Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. Stand Genom Sci 2:117–134
- Ayers M, Sampaleanu LM, Tammam S, Koo J, Harvey H, Howell PL, Burrows LL (2009) PilM/N/O/P proteins form an inner membrane complex that affects the stability of the *Pseudomonas aeruginosa* type IV pilus secretin. J Mol Biol 394:128–142
- Bankevich A et al (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477
- Boesch C, Trček J, Sievers M, Teuber M (1998) *Acetobacter intermedium*, sp. nov. Syst Appl Microbiol 21:220–229
- Borriello SP, Davies HA, Kamiya S, Reed PJ, Seddon S (1990) Virulence factors of *Clostridium difficile*. Rev Infect Dis 12(Suppl 2):S185–S191
- Bushnell B (2014) BBTools software package. <http://sourceforge.net/projects/bbmap>
- Chen IM, Markowitz VM, Palaniappan K, Szeto E, Chu K, Huang J, Ratner A, Pillay M, Hadjithomas M, Huntemann M, Mikhailova N, Ovchinnikova G, Ivanova NN, Kyrpides NC (2016) Supporting Community annotation and user collaboration in the Integrated Microbial Genomes (IMG) system. BMC Genom 17:307
- Chen IA, Markowitz VM, Chu K, Palaniappan K, Szeto E, Pillay M, Ratner A, Huang J, Andersen E, Huntemann M, Varghese N, Hadjithomas M, Tennessen K, Nielsen T, Ivanova NN, Kyrpides NC (2017) IMG/M: integrated genome and metagenome comparative data analysis system. Nucleic Acids Res 45(D1):D507–D516
- Craig L, Pique ME, Tainer JA (2004) Type IV pilus structure and bacterial pathogenicity. Nat Rev Microbiol 2:363–378
- Felsenstein J (2002) PHYLIP (Phylogeny Inference Package) version 3.6. Department of Genetics, University of Washington, Seattle
- Fives-Taylor PM, Thompson DW (1985) Surface properties of *Streptococcus sanguis* FW213 mutants nonadherent to saliva-coated hydroxyapatite. Infect Immun 47:752–759
- Gerhardt P, Murray RGE, Wood WA (1994) Methods for general and molecular bacteriology. American Society for Microbiology, Washington
- Han TY, Tong XM, Wang YW, Wang HM, Chen XR, Kong DL, Guo X, Ruan ZY (2015) *Paenibacillus populi* sp. nov., a novel bacterium isolated from the rhizosphere of *Populus alba*. Antonie Van Leeuwenhoek 108:659–666
- Huntemann M, Ivanova NN, Mavromatis K et al (2015) The standard operating procedure of the DOE-JGI Microbial genome annotation pipeline (MGAP v4). Stand Genomic Sci 10:86
- Imam S, Chen Z, Roos DS, Pohlschröder M (2011) Identification of surprisingly diverse type IV Pili, across a broad range of gram-positive bacteria. PLoS ONE 6(12):e28919
- Kim KK, Lee KC, Yu H, Ryoo S, Park Y, Lee JS (2010) Int J Syst Evol Microbiol 60:2371–2376
- Lee FL, Kuo HP, Tai CJ, Yokota A, Lo CC (2007) *Paenibacillus taiwanensis* sp. nov., isolated from soil in Taiwan. Int J Syst Evol Microbiol 57:1351–1354
- Ludwig W, Schleifer KH, Whitman WB (2009) Family IV. *Paenibacillaceae* fam. nov. In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey F, Schleifer KH,

- Whitman WB (eds) *Bergey's manual of systematic bacteriology*, vol 3, 2nd edn. Springer, New York, p 269
- Mah JH, Chang YH, Hwang HJ (2008) *Paenibacillus tyraminigenes* sp. nov. isolated from Myeolchi-jeotgal, a traditional Korean salted and fermented anchovy. *Int J Food Microbiol* 127:209–214
- Meier-Kolthoff JO, Alexander AF, Hans-Peter K, Markus K (2013) Genome sequence-based specie delimitation with confidence intervals and improved distance functions. *BMC Bioinform* 14:60
- Melville S, Craig L (2013) Type IV Pili in gram-positive bacteria. *Microbiol Mol Biol Rev* 77(3):323–341
- Nakamura LK (1996) *Paenibacillus apiarius* sp. nov. *Int J Syst Bacteriol* 46:688–693
- Page RDM (2000) TreeView version 1.6.6. <http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>. Accessed 20 Jan 2005.
- Pati A, Ivanova NN, Mikhailova N, Ovchinnikova G, Hooper SD, Lykidis A, Kyrpides NC (2010) GenePRIMP: a GENE PRediction IMprovement Pipeline for Prokaryotic genomes. *Nat Methods* 7(6):455–457
- Priest FG (2015) *Paenibacillus*. *Bergey's manual of systematics of archaea and bacteria*. Wiley, Hoboken, pp 1–40
- Richter M, Rosselló-Móra R (2009) Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 106:19126–19131
- Romanenko LA, Tanaka N, Svetashev VI, Kalinovskaya NI (2013) *Paenibacillus profundus* sp. nov., a deep sediment bacterium that produces isocoumarin and peptide antibiotics. *Arch Microbiol* 195:247–254
- Rosselló-Móra R, Trujillo ME, Sutcliffe IC (2017) Introducing a digital protologue: a timely move towards a database-driven systematics of archaea and bacteria. *Antonie Van Leeuwenhoek* 110:455–456
- Roux V, Raoult D (2004) *Paenibacillus massiliensis* sp. nov., *Paenibacillus sanguinis* sp. nov. and *Paenibacillus timonensis* sp. nov., isolated from blood cultures. *Int J Syst Evol Microbiol* 54:1049–1054
- Roux V, Fenner L, Raoult D (2008) *Paenibacillus provencensis* sp. nov., isolated from human cerebrospinal fluid, and *Paenibacillus urinalis* sp. nov., isolated from human urine. *Int J Syst Evol Microbiol* 58:682–687
- Saha P, Mondal AK, Mayilraj S, Krishnamurthi S, Bhattacharya A, Chakrabarti T (2005) *Paenibacillus assamensis* sp. nov., a novel bacterium isolated from a warm spring in Assam, India. *Int J Syst Evol Microbiol* 55:2577–2581
- Schumann P (2011) Peptidoglycan structure. In: Rainey FA, Oren A (eds) *Taxonomy of prokaryotes*. Academic Press, Chennai, pp 102–129
- Shida O, Takagi H, Kadowaki K, Nakamura LK, Komagata K (1997) Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, *Bacillus kobensis*, and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. *Int J Syst Bacteriol* 47:289–298
- Sitdhapol J, Paek J, Sin Y, Park IS, Thamacharoensuk T, Wannissorn B, Tanasupawat S, Chang YH (2016) *Paenibacillus cathormii* sp. nov., isolated from tree bark. *Int J Syst Evol Microbiol* 66:1187–1192
- Stackebrandt E, Ebers J (2006) Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* 33:152–155
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX interface: flexible strategies for multiple sequence alignment aided by quality analysis tool. *Nucleic Acids Res* 25:4876–4882
- Tindall BJ (1990a) A comparative study of the lipid composition of *Halobacterium saccharovororum* from various sources. *Syst Appl Microbiol* 13:128–130
- Tindall BJ (1990b) Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 66:199–202
- Trček J, Raspor P, Teuber M (2000) Molecular identification of *Acetobacter* isolates from submerged vinegar production, sequence analysis of plasmid pJK2-1 and application in development of a cloning vector. *Appl Microbiol Biotechnol* 53:289–295
- Varghese N, Mukherjee S, Ivanova NN, Konstantidinis K, Mavrommatis K, Kyrpides NC, Pati A (2015) Microbial species delineation using whole genome sequences. *Nucleic Acids Res* 43(14):6761–6771
- Whitman WB, Woyke T, Klenk HP, Zhou Y, Lilburn TG, Beck BJ, De Vos P, Vandamme P, Eisen JA, Garrity G, Hugenholtz P, Kyrpides NC (2015) Genomic encyclopedia of bacterial and archaeal type strains, phase III: the genomes of soil and plant-associated and newly described type strains. *Stand Genom Sci* 10:26