

Bacillus marisflavi sp. nov. and *Bacillus aquimaris* sp. nov., isolated from sea water of a tidal flat of the Yellow Sea in Korea

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Two Gram-positive or Gram-variable, endospore-forming, moderately halophilic rods (strains TF-11^T and TF-12^T) were isolated from a tidal flat of the Yellow Sea in Korea and were subjected to a polyphasic taxonomic study. Strains TF-11^T and TF-12^T had cell-wall peptidoglycan based on *meso*-diaminopimelic acid. The predominant menaquinone found in the two strains was MK-7. The cellular fatty acid profiles of both strains contained large amounts of branched and saturated fatty acids. The major fatty acids were anteiso-C_{15:0} and iso-C_{15:0}. The DNA G + C contents of strains TF-11^T and TF-12^T were respectively 49 and 38 mol%. Phylogenetic analysis based on 16S rDNA sequences showed that strains TF-11^T and TF-12^T fell within the radiation of the cluster comprising *Bacillus* species. The level of 16S rDNA sequence similarity between strains TF-11^T and TF-12^T was 98.3%. Strains TF-11^T and TF-12^T exhibited levels of 16S rDNA sequence similarity of less than 96.0 and 96.3%, respectively, to *Bacillus* species. The mean level of DNA–DNA relatedness between the two strains was approximately 7%. On the basis of phenotypic and phylogenetic data and genomic distinctiveness, strains TF-11^T and TF-12^T should be placed in the genus *Bacillus* as two distinct novel species, for which the names *Bacillus marisflavi* sp. nov. (type strain TF-11^T = KCCM 41588^T = JCM 11544^T) and *Bacillus aquimaris* sp. nov. (type strain TF-12^T = KCCM 41589^T = JCM 11545^T) are proposed.

INTRODUCTION

Moderately halophilic or halotolerant, Gram-positive or -variable, endospore-forming rods have been commonly isolated from marine environments and related regions or materials (Ventosa *et al.*, 1998; Wainø *et al.*, 1999; Yoon *et al.*, 2001a). In the course of screening micro-organisms present in a tidal flat of the Yellow Sea in Korea, many moderately halophilic or halotolerant, endospore-forming rods have been isolated and characterized taxonomically. In this study, two moderately halophilic, rod-shaped, endospore-forming strains (TF-11^T and TF-12^T) isolated

from this environment are described. Most Gram-positive or Gram-variable, endospore-forming rods with moderately halophilic or halotolerant properties have previously been assigned to the genus *Bacillus*, e.g. *Bacillus dipsosauri* (Lawson *et al.*, 1996), *Bacillus halodurans* (Nielsen *et al.*, 1995), *Bacillus halophilus* (Ventosa *et al.*, 1989), *Bacillus marinus* (Rüger, 1983; Rüger & Richter, 1979), *Bacillus marismortui* (Arahal *et al.*, 1999), *Bacillus pantothenicus* (Proom & Knight, 1950), *Bacillus salexigens* (Garabito *et al.*, 1997) and others (Claus & Berkeley, 1986). Recently, innovations in the study of bacterial systematics have aided the reclassification of many species assigned to the genus *Bacillus* (Ash *et al.*, 1993; Heyndrickx *et al.*, 1998; Shida *et al.*, 1996; Wisotzkey *et al.*, 1992). Many moderately halophilic or halotolerant *Bacillus* species have also been reclassified as members of new genera or transferred to other genera. *Bacillus dipsosauri* has been transferred to *Gracilibacillus*, together with the description of a novel genus and species, *Gracilibacillus halotolerans* (Wainø *et al.*,

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The GenBank accession numbers for the 16S rDNA sequences of strains TF-11^T and TF-12^T are AF483624 and AF483625.

An extended phylogenetic tree is available as supplementary material in IJSEM Online.

1999). *Bacillus salexigens* has been reclassified as *Salibacillus salexigens* (Wainø *et al.*, 1999) and *Bacillus marinus* has been reclassified as *Marinibacillus marinus* (Yoon *et al.*, 2001b). *Bacillus marismortui* has been transferred to the genus *Salibacillus* as *Salibacillus marismortui* (Arahal *et al.*, 2000). Interestingly, many recently isolated bacilli with moderately halophilic or halotolerant properties have been identified as members of related new genera rather than members of the genus *Bacillus* (Lu *et al.*, 2001; Schlesner *et al.*, 2001; Spring *et al.*, 1996; Wainø *et al.*, 1999; Yoon *et al.*, 2001b). These facts indicate the importance of a polyphasic approach for determination of the exact taxonomic positions of moderately halophilic or halotolerant, endospore-forming rods. Accordingly, the aim of the present study was to determine the exact taxonomic status of strains TF-11^T and TF-12^T using a combination of phenotypic properties, phylogenetic analysis based on 16S rDNA sequences and genomic relatedness. On the basis of the data presented below, strains TF-11^T and TF-12^T should be placed in the genus *Bacillus* as two distinct novel species, *Bacillus marisflavi* sp. nov. and *Bacillus aquimaris* sp. nov., respectively.

METHODS

Bacterial strains and cultural conditions. Strains TF-11^T and TF-12^T were isolated from sea water of a tidal flat of the Yellow Sea in Korea by the dilution plating technique on marine agar (MA) (Difco). Cell biomass of strains TF-11^T and TF-12^T for analyses of the cell wall and menaquinones and for DNA extraction was obtained from marine broth (MB) (Difco) cultures at 30 °C. The two strains were cultivated on a horizontal shaker at 150 r.p.m. and broth cultures were checked for purity by microscopic examination before being harvested by centrifugation. For fatty acid methyl ester analysis, cell mass was obtained from agar plates after cultivation for 2 days at 30 °C on MA.

Morphological and physiological characterization. Cell morphology was examined by light microscopy and TEM. Flagellum type was examined by TEM using cells from exponentially growing cultures. The cells were negatively stained with 1% (w/v) phosphotungstic acid and, after air drying, the grids were examined using a model CM-20 TEM (Philips). The Gram reaction was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Catalase activity was determined by bubble production in a 3% (v/v) hydrogen peroxide solution. Oxidase activity was determined by oxidation of 1% *p*-aminodimethylaniline oxalate. Urease activity was determined as described by Cowan & Steel (1965), carried out in the presence and absence of 3% (w/v) NaCl. Hydrolysis of casein and starch was determined as described by Cowan & Steel (1965). Hydrolysis of gelatin was determined as described by Cowan & Steel (1965), in the presence and absence of 3% (w/v) NaCl. Hydrolysis of aesculin and Tween 80 was determined as described by Cowan & Steel (1965) or performed on MA with the concentration of substrates described previously (Cowan & Steel, 1965). Nitrate reduction was determined as described by Lanyi (1987), in the presence and absence of 3% (w/v) NaCl. Hydrolysis of hypoxanthine, tyrosine and xanthine was examined on MA with substrate concentrations described previously (Cowan & Steel, 1965). Acid production from carbohydrates was determined as described by Leifson (1963) and using the API 50CH system (bioMérieux). Cell mass of strains TF-11^T and TF-12^T was suspended in 2 ml artificial sea water which contained (l⁻¹ distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g

MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Levring, 1946). This suspension was added to API 50CHB medium. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber with MA that had been prepared anaerobically. Growth at various NaCl concentrations was investigated on MA or in MB. Growth at various temperatures was measured on MA at 4–55 °C.

Isolation of DNA. Chromosomal DNA was isolated and purified according to Yoon *et al.* (1996), with the exception that ribonuclease T1 was used together with ribonuclease A.

Chemotaxonomic characterization. The isomer type of diamino acid of the cell-wall peptidoglycan was determined by the method of Komagata & Suzuki (1987). Menaquinones were analysed as described previously (Komagata & Suzuki, 1987) using reversed-phase HPLC. For quantitative analysis of cellular fatty acid compositions, a loop of cell mass was harvested and fatty acid methyl esters were prepared and identified following the instructions of the Microbial Identification system (MIDI).

Determination of G+C content. The G+C content was determined by the method of Tamaoka & Komagata (1984). DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

DNA–DNA hybridization. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. Of the values obtained, the highest and lowest values in each sample were excluded. DNA–DNA relatedness values are expressed as the mean of the remaining three values.

16S rDNA sequencing and phylogenetic analysis. 16S rDNA was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). The PCR product was purified with a QIAquick PCR purification kit (Qiagen). Sequencing of the purified 16S rDNA was performed using an ABI PRISM BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems) as recommended by the manufacturer. The purified sequencing reaction mixtures were electrophoresed automatically using an Applied Biosystems model 377 automatic DNA sequencer. Alignment of sequences was carried out using CLUSTAL W software (Thompson *et al.*, 1994). Gaps at the 5' and 3' ends of the alignment were omitted from further analysis. Phylogenetic trees were inferred using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods contained within the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices for the neighbour-joining method were calculated using the algorithm of Jukes & Cantor (1969) with the program DNADIST. The stability of relationships was assessed by a bootstrap analysis based on 1000 resampling of the neighbour-joining dataset using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package.

RESULTS AND DISCUSSION

Morphology

Strain TF-11^T was Gram-positive but changed to Gram-variable as the cultures aged. Strain TF-12^T was Gram-variable. Cells of strain TF-11^T and TF-12^T were rods, approximately 0.6–0.8 × 1.5–3.5 μm and 0.5–0.7 × 1.2–3.5 μm, respectively, after 3 days cultivation at 30 °C on MA (Fig. 1). Strains TF-11^T and TF-12^T were respectively

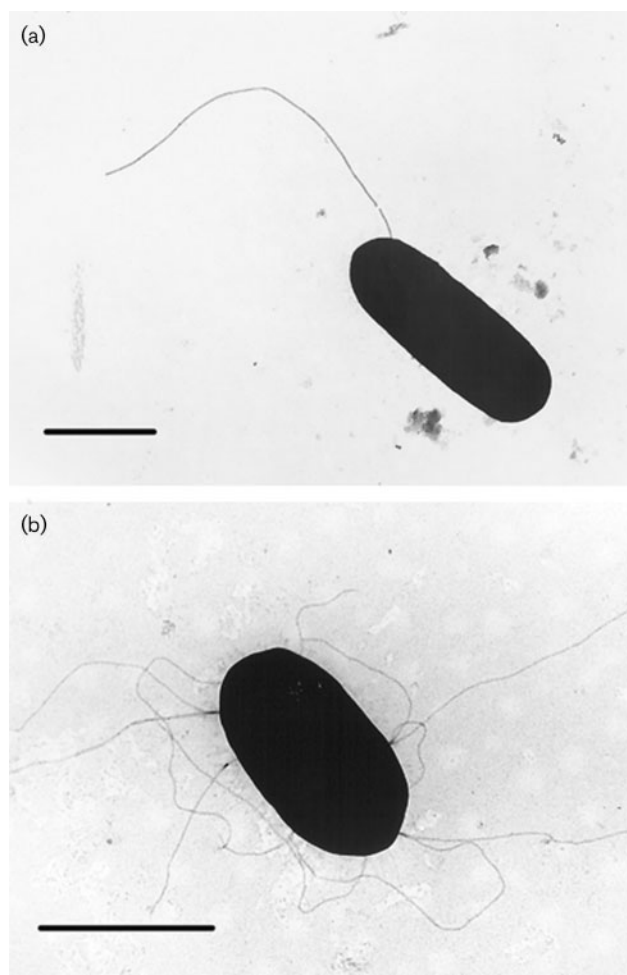


Fig. 1. TEM of strains TF-11^T (a) and TF-12^T (b) from 3-day-old cultures grown on MA. Bars, 1 µm.

motile by means of a single polar flagellum and peritrichous flagella. Strain TF-11^T had ellipsoidal endospores observed at subterminal or central positions in swollen sporangia. Strain TF-12^T had ellipsoidal endospores produced centrally in large, swollen sporangia. Colonies of strain TF-11^T were smooth, circular to slightly irregular, slightly raised, pale yellow in colour and 2–4 mm in diameter after 3 days growth at 30 °C on MA. Colonies of strain TF-12^T were circular to slightly irregular, slightly raised, pale orange-yellow in colour and 2–4 mm in diameter after 3 days growth at 30 °C on MA.

Cultural and physiological characteristics

Strains TF-11^T and TF-12^T grew optimally at 30–37 °C. Strain TF-11^T grew at 10 and 47 °C, but not at 4 °C or above 48 °C. Strain TF-12^T grew at 10 and 44 °C, but not at 4 °C or above 45 °C. Strains TF-11^T and TF-12^T respectively grew optimally at pH 6.0–8.0 and pH 6.0–7.0. Strain TF-11^T grew at pH 4.5 and 9.0, but strain TF-12^T did not. Strain TF-11^T did not grow at pH 4.0. Strains TF-11^T

and TF-12^T grew optimally in the presence of 2–5 % (w/v) NaCl. In the absence of NaCl, strain TF-11^T grew relatively well, but strain TF-12^T showed poor growth. Strains TF-11^T and TF-12^T did not grow in the presence of more than 17 and 19 % (w/v) NaCl, respectively. The two strains did not grow under anaerobic conditions on MA. Both strains had catalase activity, but not oxidase or urease activities. Casein was hydrolysed, but no hydrolysis of hypoxanthine, tyrosine or xanthine was observed. Aesculin was hydrolysed by strain TF-11^T, but not by strain TF-12^T. Starch and Tween 80 were hydrolysed by strain TF-12^T, but not by strain TF-11^T. The phenotypic properties of strains TF-11^T and TF-12^T are summarized in Table 1.

Chemotaxonomic characteristics and DNA base composition

Strains TF-11^T and TF-12^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The predominant menaquinone was unsaturated menaquinone with seven isoprene units (MK-7). Both strains had cellular fatty acid profiles containing large amounts of branched and saturated fatty acids (Table 2). Strains TF-11^T and TF-12^T contained anteiso-C_{15:0} and iso-C_{15:0} as the major fatty acids (Table 2). At present in bacterial systematics, chemotaxonomic differentiation is not necessarily possible among some aerobic or facultatively anaerobic, rod-shaped, endospore-forming genera. Nevertheless, the chemotaxonomic properties obtained for strains TF-11^T and TF-12^T were most similar to those of the genus *Bacillus* (Shida *et al.*, 1997). The genomic DNA G+C contents of strains TF-11^T and TF-12^T were respectively 49 and 38 mol%.

Phylogenetic analysis

The 16S rDNA sequences of strains TF-11^T and TF-12^T determined in this study comprised 1506 and 1507 nt, respectively, representing approximately 96 % of the *Escherichia coli* 16S rRNA sequence. The level of 16S rDNA sequence similarity between strains TF-11^T and TF-12^T was 98.3 %. A phylogenetic tree, generated using the neighbour-joining algorithm, showed that strains TF-11^T and TF-12^T both fell within the radiation of the cluster comprising *Bacillus* species and formed a coherent cluster that is supported by a bootstrap analysis at a confidence level of 100 % (Fig. 2). This cluster joins with the phylogenetic clade comprising *Bacillus subtilis*, the type species of the genus *Bacillus* (Fig. 2). However, the clustering fidelity between the two phylogenetic groups was supported by a relatively low bootstrap resampling value of 56.4 % (Fig. 2). This tree topology was also found in trees generated with the maximum-likelihood and maximum-parsimony algorithms (not shown). Strains TF-11^T and TF-12^T exhibited levels of 16S rDNA sequence similarity of less than 96.0 and 96.3 %, respectively, to *Bacillus* species whose 16S rDNA sequences are known.

Table 1. Phenotypic properties of strains TF-11^T and TF-12^T

+, Positive; -, negative; W, weakly positive; V, variable. Both strains were rods and had ellipsoidal endospores. Both strains were positive for catalase, hydrolysis of casein and acid production from D-fructose, D-glucose, maltose, D-ribose, sucrose and D-trehalose. Both strains were negative for oxidase, urease, hydrolysis of hypoxanthine, tyrosine and xanthine and acid production from N-acetylglucosamine, adonitol, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, L-fucose, gluconate, inulin, 2-ketogluconate, lactose, D-lyxose, myo-inositol, D-melezitose, methyl α -D-glucoside, methyl β -D-xylose, L-rhamnose, D-sorbitol, sorbose, D-tagatose, D-turanose, xylitol and L-xylose.

Character	Strain TF-11 ^T	Strain TF-12 ^T
Gram staining	+ (or v)	v
Flagellum type	Single polar	Peritrichous
Spore position	Subterminal or central	Central
Colony colour	Pale yellow	Pale orange-yellow
Growth at 45 °C	+	-
Optimal pH for growth	6.0-8.0	6.0-7.0
Growth at pH:		
4.5	+	-
9.0	+	-
Growth in NaCl at (% w/v):		
0	+	W
17	-	+
Hydrolysis of:		
Aesculin	+	-
Tween 80	-	+
Starch	-	+
Acid production from:		
Aesculin	+	-
Arbutin	+	-
D-Cellobiose	+	-
D-Galactose	W	-
Gentiobiose	+	-
Glycerol	+	-
Glycogen	-	+
5-Ketogluconate	-	+
D-Mannitol	+	-
D-Mannose	+	-
Melibiose	+	-
Methyl α -D-mannoside	+	-
D-Raffinose	W	-
Salicin	+	-
Stachyose	+	-
Starch	-	+
D-Xylose	+	-
Major fatty acids	anteiso-C _{15:0} , iso-C _{15:0}	iso-C _{15:0} , anteiso-C _{15:0}
G + C content (mol%)	49	38

DNA-DNA relatedness

DNA-DNA hybridization was performed to determine the genomic relatedness between strains TF-11^T and TF-12^T. Strains TF-11^T and TF-12^T exhibited two independent levels of DNA-DNA relatedness, of 5.1 and 8.8%, when each of their DNAs was used separately as the labelled DNA probe. These data indicate that strains TF-11^T and TF-12^T are members of two different genomic species of the genus *Bacillus* (Wayne *et al.*, 1987).

Conclusions

16S rDNA sequence comparison showed that strains TF-11^T and TF-12^T exhibited closest phylogenetic affinities to *Bacillus* species. Phylogenetic analysis based on 16S rDNA sequences shows that the two strains form distinct phylogenetic lineages within the evolutionary radiation enclosed by the genus *Bacillus* (Fig. 2). The results obtained in the morphological and chemotaxonomic analyses are consistent with the results of 16S rDNA

Table 2. Cellular fatty acid profiles of strains TF-11^T and TF-12^T

Values are percentages of total fatty acids. —, Not detected; i, iso; ai, anteiso.

Fatty acid	TF-11 ^T	TF-12 ^T
i-C _{13:0}	—	0.4
i-C _{14:0}	9.1	6.5
i-C _{14:0} 3-OH	2.0	—
C _{14:0}	1.7	0.9
i-C _{15:0}	22.9	46.9
i-C _{15:0} 3-OH	6.1	—
i-C _{15:1}	—	0.7
ai-C _{15:0}	27.7	22.2
C _{15:0}	1.1	0.5
C _{15:0} 2-OH	5.5	—
i-C _{16:0}	7.7	4.2
C _{16:0}	1.0	0.5
i-C _{16:0} 3-OH	1.5	—
C _{16:1} ω7c alcohol	2.5	8.4
C _{16:1} ω11c	—	1.3
i-C _{17:0}	1.2	0.8
i-C _{17:1} ω10c	—	1.8
ai-C _{17:0}	7.1	2.3
C _{17:0} 2-OH	1.2	—
Summed feature 4*	1.8	2.9

*Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 4 contains one or more of iso-C_{17:1} I and/or anteiso-C_{17:1} B.

sequence comparison and phylogenetic inference (Table 1). Strains TF-11^T and TF-12^T could be clearly differentiated from each other in their morphological and physiological characteristics. There are distinct morphological differences between them, including colony colour, flagellum type and endospore position. There are also some physiological differences between strains TF-11^T and TF-12^T, such as tolerance of NaCl, temperature and pH for growth, their ability to hydrolyse some substrates and acid production from carbohydrates (Table 1). It is noteworthy that strains TF-11^T and TF-12^T show distinct differences in G+C content and in proportions of some fatty acids, particularly iso- and anteiso-C_{15:0} (Table 2). The mean level of DNA–DNA relatedness between the two strains is approximately 7%. This genomic relatedness provides decisive evidence that strains TF-11^T and TF-12^T are members of different genomic species (Wayne *et al.*, 1987). The 16S rDNA sequence similarity values of strains TF-11^T and TF-12^T to the type strains of other *Bacillus* species are respectively less than 96.0 and 96.3%. There are widely accepted criteria for delineating species in current bacteriology, stating that strains with a level of DNA relatedness of less than 70% or with more than 3% difference in 16S rDNA sequences are considered to represent different species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Sequence similarity

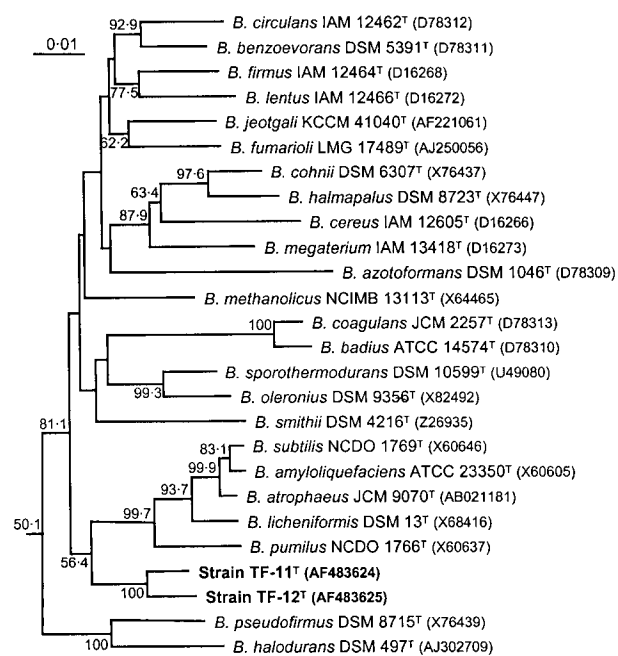


Fig. 2. Neighbour-joining tree showing the phylogenetic positions of strains TF-11^T and TF-12^T and representatives of some other related taxa based on 16S rDNA sequences. Bar, 0.01 substitutions per nucleotide position. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branch-points. An extended phylogenetic tree is available as supplementary material in IJSEM Online.

values obtained for strains TF-11^T and TF-12^T and the type strains of all *Bacillus* species with validly published names are low enough to categorize strains TF-11^T and TF-12^T as two distinct species within the genus *Bacillus*. On the basis of the data described above, strains TF-11^T and TF-12^T should be placed in the genus *Bacillus* as two distinct novel species, for which the names *Bacillus marisflavi* sp. nov. and *Bacillus aquimaris* sp. nov., respectively, are proposed.

Description of *Bacillus marisflavi* sp. nov.

Bacillus marisflavi (ma.ris fla'vi. L. gen. neut. n. *maris* of the sea; L. masc. adj. *flavus* yellow; N.L. gen. masc. n. *marisflavi* of the Yellow Sea).

Cells are aerobic rods, 0.6–0.8 × 1.5–3.5 μm. Gram-positive, but Gram-variable in older cultures. Motile by means of a single polar flagellum. Central or subterminal ellipsoidal endospores are observed in swollen sporangia. Colonies are smooth, circular to slightly irregular, slightly raised, pale yellow in colour and 2–4 mm in diameter after 3 days at 30 °C on MA. Optimal growth temperature is 30–37 °C. Growth occurs at 10 and 47 °C, but not at 4 or above 48 °C. Optimal growth pH is 6.0–8.0. Growth is observed at pH 4.5, but not at pH 4.0. Optimal growth occurs in the presence of 2–5% (w/v) NaCl. Growth occurs in the presence of 0–16% (w/v) NaCl. Growth does not

occur under anaerobic conditions on MA. Catalase-positive. Oxidase- and urease-negative. Aesculin and casein are hydrolysed. Hypoxanthine, starch, Tween 80, tyrosine and xanthine are not hydrolysed. Acid is produced from D-cellobiose, D-fructose, D-glucose, maltose, D-mannitol, D-mannose, melibiose, D-ribose, stachyose, sucrose, D-trehalose and D-xylose and produced weakly from D-galactose and D-raffinose. Acid is not produced from adonitol, L-arabinose, lactose, D-melezitose, *myo*-inositol, L-rhamnose or D-sorbitol. Results using the API 50CHB system show that acid is produced from aesculin, arbutin, gentiobiose, glycerol, methyl α -D-mannoside and salicin, but not from *N*-acetylglucosamine, amygdalin, D-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, D-fucose, L-fucose, gluconate, glycogen, inulin, 2-ketogluconate, 5-ketogluconate, D-lyxose, methyl α -D-glucoside, methyl β -D-xylose, sorbose, starch, D-tagatose, D-turanose, xylitol or L-xylose. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid. The predominant menaquinone is MK-7. The major fatty acids are anteiso-C_{15:0} and iso-C_{15:0}. The G+C content of the type strain is 49 mol% (determined by HPLC).

The type strain, TF-11^T (=KCCM 41588^T=JCM 11544^T), was isolated from sea water of a tidal flat of the Yellow Sea in Korea.

Description of *Bacillus aquimaris* sp. nov.

Bacillus aquimaris (a.qui.ma'ris. L. n. *aqua* water; L. gen. n. *maris* of the sea; N.L. gen. n. *aquimaris* of the water of the sea).

Cells are aerobic rods, 0.5–0.7 × 1.2–3.5 µm. Gram-variable. Motile by means of peritrichous flagella. Central ellipsoidal endospores are observed in large, swollen sporangia. Colonies are circular to slightly irregular, slightly raised, pale orange-yellow in colour and 2–4 mm in diameter after 3 days at 30 °C on MA. Optimal growth temperature is 30–37 °C. Growth occurs at 10 and 44 °C, but not at 4 or above 45 °C. Optimal growth pH is 6.0–7.0; no growth is observed at pH 9.0 or 4.5. Optimal growth occurs in the presence of 2–5% (w/v) NaCl. Growth is poor in the absence of NaCl, but occurs in the presence of up to 18% (w/v) NaCl. Growth does not occur under anaerobic conditions on MA. Catalase-positive. Oxidase- and urease-negative. Casein, starch and Tween 80 are hydrolysed. Aesculin, hypoxanthine, tyrosine and xanthine are not hydrolysed. Acid is produced from D-fructose, D-glucose, maltose, D-ribose, sucrose and D-trehalose. Acid is not produced from adonitol, L-arabinose, D-cellobiose, D-galactose, lactose, D-mannitol, D-mannose, D-melezitose, melibiose, *myo*-inositol, D-raffinose, L-rhamnose, D-sorbitol, stachyose or D-xylose. Results using the API 50CHB system show that acid is produced from glycogen, 5-ketogluconate and starch, but not from *N*-acetylglucosamine, aesculin, amygdalin, D-arabinose, D-arabitol, L-arabitol, arbutin, dulcitol, erythritol, D-fucose, L-fucose, gentiobiose, gluconate, glycerol, inulin, 2-ketogluconate, D-lyxose, methyl

α -D-glucoside, methyl α -D-mannoside, methyl β -D-xylose, salicin, sorbose, D-tagatose, D-turanose, xylitol or L-xylose. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid. The predominant menaquinone is MK-7. The major fatty acids are iso-C_{15:0} and anteiso-C_{15:0}. The G+C content of the type strain is 38 mol% (determined by HPLC).

The type strain, TF-12^T (=KCCM 41589^T=JCM 11545^T), was isolated from sea water of a tidal flat of the Yellow Sea in Korea.

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