Bacillus cibi sp. nov., isolated from jeotgal, a traditional Korean fermented seafood

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A Gram-variable, motile, endospore-forming, halotolerant bacillus, strain JG-30^T, was isolated from the traditional Korean fermented seafood jeotgal, and was subjected to a polyphasic taxonomic study. This organism grew optimally at 37 °C and in the presence of 0–1 % (w/v) NaCl. 16S rRNA gene sequence analysis showed that strain JG-30^T forms a distinct phylogenetic lineage within the evolutionary radiation encompassed by the genus *Bacillus*. Strain JG-30^T was characterized chemotaxonomically as having cell-wall peptidoglycan based on *meso*-diaminopimelic acid, MK-7 as the predominant menaquinone and iso-$C_{15:0}$ and iso-$C_{14:0}$ as the major fatty acids. The DNA G+C content was 45 mol%. Strain JG-30^T exhibited levels of 16S rRNA gene sequence similarity of less than 95·7 % to *Bacillus* species with validly published names. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain JG-30^T (=KCTC 3880^T=DSM 16189^T) was classified within the genus *Bacillus* as a novel species, for which the name *Bacillus cibi* sp. nov. is proposed.

Studies on the microbiota of jeotgal, a traditional Korean fermented seafood, have shown that the majority of isolates are Gram-positive or Gram-variable, endospore-forming bacilli (Yoon *et al.*, 2001a, b). Some new genera or species have been isolated from jeotgal (Yoon *et al.*, 2001a, b, 2003a, b). In this study, we report on the taxonomic characterization of a halotolerant, orange/yellow-pigmented *Bacillus*-like bacterial strain, JG-30^T, which was isolated from jeotgal.

Strain JG-30^T was isolated by means of the usual dilution plating technique on marine agar 2216 (MA; Difco) at 30 °C. The cell morphology was examined by light microscopy (model E600; Nikon) and transmission electron microscopy. The flagellum type was also examined by transmission electron microscopy (model CM-20; Philips) using cells from exponentially growing cultures. The Gram reaction was determined using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber with MA that had been prepared anaerobically using nitrogen. Growth in the absence of NaCl was investigated in trypticase soy broth without NaCl. Growth at various NaCl concentrations was investigated in marine broth 2216 (MB; Difco) or trypticase soy broth. Growth at various temperatures (4–50 °C) was measured on MA. Catalase and oxidase activities and the hydrolysis of casein, gelatin, hypoxanthine, starch, Tween 80, tyrosine and xanthine were tested for as described by Cowan & Steel (1965). Hydrolysis of aesculin, Voges–Proskauer and methyl red reactions and nitrate reduction were determined as described by Lanyi (1987). H_2S production was tested for as described previously (Bruns *et al.*, 2001). Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of substrates as sole carbon and energy sources was tested as described by Baumann & Baumann (1981) using supplementation with 2 % (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957) and 1 % (v/v) vitamin solution (Staley, 1968). Enzyme activity was determined by using the API ZYM system (bioMérieux). Other physiological and biochemical tests were performed with the API 20E system (bioMérieux).

Cell biomass for analyses of the cell-wall peptidoglycan and isoprenoid quinones and for DNA extraction was obtained from cultivation in MB at 37 °C. The isomer type of the diamino acid of the cell-wall peptidoglycan was determined by the method of Komagata & Suzuki (1987). Isoprenoid quinones were extracted and analysed as described previously (Komagata & Suzuki, 1987) using reversed-phase HPLC. For fatty acid methyl ester analysis, a loop of cell mass was harvested from agar plates after cultivation for 2 days at 37 °C on MA. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Chromosomal DNA was isolated and purified according to the method described previously (Yoon *et al.*, 1996), with the exception that ribonuclease T1 was used together with ribonuclease A. The DNA G+C content was

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JG-30^T is AY550276.

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determined by the method of Tamaoka & Komagata (1984), with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. The 16S rRNA gene was amplified by a PCR using two universal primers, as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003a).

The morphological, physiological and biochemical characteristics of strain JG-30^T are shown in Table 1 or are given in the species description (see below). Strain JG-30^T contained *meso*-diaminopimelic acid as the diamino acid in the cell-wall peptidoglycan and it had an unsaturated menaquinone with seven isoprene units (MK-7) as the predominant isoprenoid quinone. The cellular fatty acid profile showed a large amount of branched fatty acids; the major components were iso-$C_{15:0}$ (31·8 %), iso-$C_{14:0}$ (26·7 %), iso-$C_{16:0}$ (15·6 %) and anteiso-$C_{15:0}$ (15·4 %). The DNA G + C content of strain JG-30^T was 45 mol%. An almost-complete 16S rRNA gene sequence of strain JG-30^T comprising 1493 nt (approx. 96 % of the *Escherichia coli* 16S rRNA sequence) was determined directly after PCR amplification. Phylogenetic analyses based on 16S

Table 1. Phenotypic characteristics of *Bacillus cibi* sp. nov. and some related *Bacillus* species

Species: 1, *B. cibi* sp. nov.; 2, *B. cohnii*, data from Spanka & Fritze (1993) and Suresh *et al.* (2004); 3, *B. halmapalus*, data from Nielsen *et al.* (1995) and Suresh *et al.* (2004); 4, *B. cereus*, data from Claus & Berkeley (1986) and Priest *et al.* (1988); 5, *B. simplex*, data from Priest *et al.* (1988) and De Clerck *et al.* (2004); 6, *B. megaterium*, data from Claus & Berkeley (1986), Reva *et al.* (2002) and Täubel *et al.* (2003); 7, *B. psychrosaccharolyticus*, data from Priest *et al.* (1988) and Suresh *et al.* (2004); 8, *B. fastidiosus*, data from Claus & Berkeley (1986); 9, *B. jeotgali*, data from Yoon *et al.* (2001a). +, Positive reaction; −, negative reaction; ND, not determined; NG, no growth; V, variable reaction. Data in parentheses are for the type strain. All species are rods and catalase-positive.

Characteristic	1	2	3	4	5	6	7	8	9
Spore shape*	E	E	E	E	E or C	E	E	E	E
Spore position†	C or S	T	P or S	C	C, S or T	C	S or T	ND	ND
Oxidase	+	+	ND	V	−	−	V	ND	−
Anaerobic growth	−	ND	−	+	−	−	−	−	+
Growth in the presence of:									
5 % (w/v) NaCl	+	+	−	+	+	+	−	+	+
10 % (w/v) NaCl	+	−	−	V	−	−	−	−	+
Growth at:									
10 °C	+	+	+	V	ND	+	+	+	+
45 °C	+	+	−	ND	ND	ND	−	ND	+
Nitrate reduction	−	+	−	+	+	−	+	−	+
Hydrolysis of:									
Aesculin	+	(−)	ND	+	−	+	+	ND	+
Casein	+	V(+)	+	+	+	+	+	−	−
Gelatin	−	+	+	+	+	+	+	−	+
Starch	+	+	+	V	+	+	+	−	+
Tween 80	+	V(+)	−	V	V	ND	+	ND	V(−)
Growth on:									
D-Galactose	+	ND	−	ND	ND	(−)	ND	ND	ND
D-Cellobiose	+	(−)	+	ND	W	(−)	ND	ND	ND
D-Mannose	+	(−)	+	ND	−	(−)	ND	ND	ND
Sucrose	+	(−)	+	ND	ND	(−)	+	ND	ND
Maltose	+	(−)	+	ND	−	(+)	ND	ND	ND
Pyruvate	+	(+)	ND	ND	ND	(−)	ND	ND	ND
Acid production from:									
D-Glucose	+	(−)	+	+	+	+	+	NG	+
Lactose	−	(−)	−	V	−	V	−	ND	−
D-Mannitol	−	(−)	−	−	V	+	V	NG	−
D-Mannose	+	(−)	−	V	V	V	+	ND	−
D-Xylose	−	(−)	+	−	−	+	+	NG	−
DNA G + C content (mol%)	45	33·9–35·0	38·6	31·7–40·1 (35·7 or 36·2)	40–41	36·5–45·0 (37·3 or 37·6)	43–44	34·3–35·1 (35·1)	41

*E, Ellipsoidal or oval; C, cylindrical.

†C, Central; S, subterminal; T, terminal; P, paraterminal.

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rRNA gene sequences showed that strain JG-30^T fell within the radiation of the cluster comprising *Bacillus* species and *Bacillus subtilis*, the type species of the genus *Bacillus* (Fig. 1). Strain JG-30^T exhibited levels of 16S rRNA gene sequence similarity to *Bacillus* species of less than 95·7 % (Fig. 1). Sequence similarities to other species included in the phylogenetic analysis were below 92·4 % (Fig. 1). The results obtained from the phenotypic and phylogenetic analyses justify a taxonomic position for strain JG-30^T as a member of the genus *Bacillus*. A widely recognized criterion for species definition in current bacteriology (Stackebrandt & Goebel, 1994) suggests that strains with more than a 3 % difference in 16S rRNA gene sequences represent different species. The phylogenetic distinctiveness of strain JG-30^T warrants separation of this strain from *Bacillus* species with validly published names (Stackebrandt & Goebel, 1994). Strain JG-30^T can be differentiated from several phylogenetically related *Bacillus* species by means of some phenotypic characteristics (Table 1). Therefore, on the basis of the data presented, strain JG-30^T should be placed in the genus *Bacillus* as a novel species, for which the name *Bacillus cibi* sp. nov. is proposed.

Description of *Bacillus cibi* sp. nov.

Bacillus cibi (ci′bi. L. n. *cibus -i* food; L. gen. n. *cibi* of food).

Cells are aerobic rods that are 0·6–0·8 × 1·5–3·5 μm in size. Gram-variable. Motile by means of peritrichous flagella. Central or subterminal ellipsoidal endospores are observed in swollen sporangia. Colonies are smooth, glistening, circular to slightly irregular, raised, orange–yellow in colour and 3–4 mm in diameter after 2 days incubation at 37 °C on MA. Optimal growth temperature is 37 °C; growth does not occur at 4 and 50 °C. Optimal pH for growth is 6·5–7·5. Growth is observed at pH 5·5 but not at pH 5·0. Growth occurs in the presence of 0–12 % (w/v) NaCl, with an optimum at 0–1 % (w/v) NaCl. Urease-negative. Hypoxanthine, tyrosine and xanthine are not hydrolysed. H_2S and indole are not produced. Voges–Proskauer and methyl-red reactions are negative. When assayed with the API 20E system, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. When assayed with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase and β-galactosidase are present, but lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. Acid is produced from D-fructose, melibiose, D-galactose, D-cellobiose, stachyose, sucrose, maltose, D-trehalose and D-raffinose. Acid is not produced from adonitol, D-sorbitol, *myo*-inositol, D-ribose, L-arabinose, D-melezitose or L-rhamnose. The following substrates are utilized for growth: D-glucose, D-fructose, D-trehalose, lactose, acetate, succinate and L-malate. D-Xylose, L-arabinose, citrate, benzoate, formate and L-glutamate are not utilized. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid. The predominant menaquinone is MK-7. The major fatty acids are iso-$C_{15:0}$ and iso-$C_{14:0}$. The DNA G + C content is 45 mol% (determined by HPLC).

The type strain, JG-30^T (= KCTC 3880^T = DSM 16189^T), was isolated from jeotgal, a traditional Korean fermented seafood.

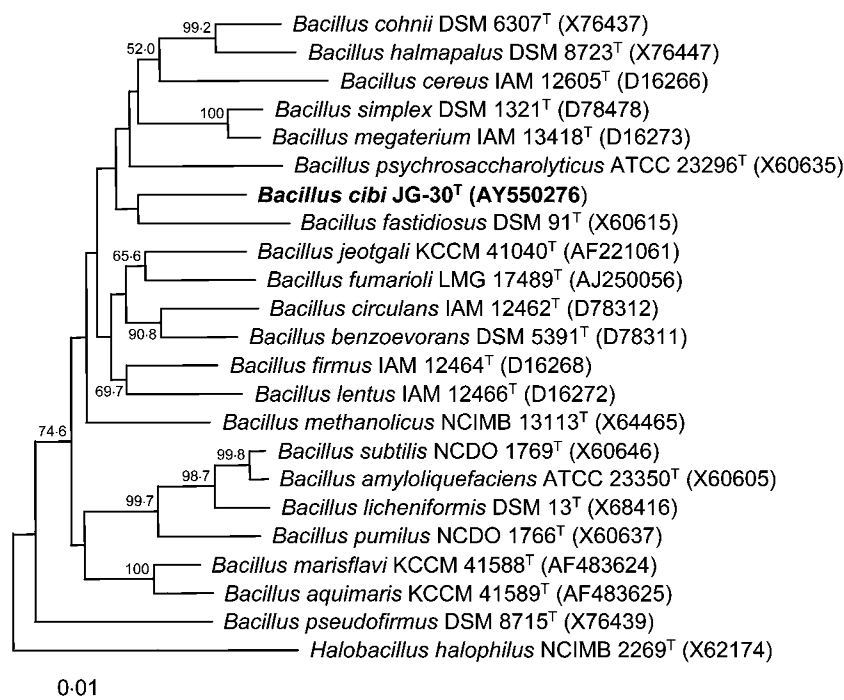

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic positions of *Bacillus cibi* JG-30^T, some *Bacillus* species and representatives of some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at the branch points. Bar, 0·01 substitution per nucleotide position.

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