Jeotgalicoccus huakuui sp. nov., a halotolerant bacterium isolated from seaside soil

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A Gram-stain-positive, oxidase- and catalase-positive, non-motile, non-spore-forming, halotolerant, coccoid bacterium, designated strain NY-2^T, was isolated from a seaside soil sample from Shandong Province, China. Strain NY-2^T was able to grow in the presence of 0–23% (w/v) NaCl and at pH 4.5–10.0 and 5–42 °C; optimum growth was observed with 3–8% (w/v) total salts and at pH 6.5–8.0 and 28–37 °C. Chemotaxonomic analyses, including fatty acid profiles, menaquinones and polar lipids, supported the affiliation of strain NY-2^T to the genus *Jeotgalicoccus*. The predominant menaquinone of strain NY-2^T was menaquinone 7 (MK-7) (100%) and the major cellular fatty acids were iso-C_{15:0} (49.0%) and anteiso-C_{15:0} (19.6%). Cellular polar lipids were phosphatidylglycerol, diphosphatidylglycerol and several unidentified phospholipids. The DNA G + C content of strain NY-2^T was 36.8 mol%. Based on 16S rRNA gene sequence analysis, strain NY-2^T formed a coherent cluster with *Jeotgalicoccus marinus* JSM 076033^T, *Jeotgalicoccus halotolerans* YKJ-101^T and *Jeotgalicoccus psychrophilus* YKJ-115^T. Phylogenetic analysis, DNA–DNA relatedness data, phenotypic characteristics and chemotaxonomic data indicated that strain NY-2^T (=CCTCC AB 208288^T =JCM 15687^T) should be classified as the type strain of a novel species of the genus *Jeotgalicoccus*, for which the name *Jeotgalicoccus huakuui* sp. nov. is proposed.

The genus *Jeotgalicoccus* was erected by Yoon *et al.* (2003) to accommodate Gram-positive, non-motile, catalase- and oxidase-positive cocci isolated from jeotgal, a traditional Korean fermented seafood. The genus *Jeotgalicoccus* forms a phylogenetically distinct line of descent that is close to, but separate from, members of the genus *Salinicoccus* and other catalase-positive, coccoid-shaped bacteria (Yoon *et al.*, 2003). Species of *Jeotgalicoccus* have been found to have halotolerant or halophilic physiological properties. At the time of writing, the genus comprises four recognized species, namely *Jeotgalicoccus halotolerans* and *Jeotgalicoccus psychrophilus* (Yoon *et al.*, 2003), *Jeotgalicoccus pinnipedialis* (Hoyles *et al.*, 2004) and *Jeotgalicoccus marinus* (Chen *et al.*, 2009). During a study of microbial resources in Shandong Province, China, a *Jeotgalicoccus*-like strain, designated NY-2^T, was isolated from a seaside soil sample. Based on the results of a polyphasic taxonomic study,

including morphological observation and physiological and chemotaxonomic analyses, the new isolate is considered to represent a novel species of the genus *Jeotgalicoccus*.

Strain NY-2^T was isolated from a seaside soil sample in Shandong Province, China. The strain grew well on Luria–Bertani (LB) medium at 30 °C. For investigation of morphological and physiological characteristics, strain NY-2^T was cultivated on LB agar at 30 °C. Cell morphology and motility were examined by light microscopy (BH-2; Olympus) and transmission electron microscopy (H-7650; Hitachi) by using cells from an exponentially growing culture. Gram staining was performed using the non-staining method described by Buck (1982). Growth at different temperatures and pH was examined by growing the strain on LB medium according to the methods of Gordon (1966, 1967). Tolerance to NaCl was tested in LB medium amended to different NaCl concentrations. Growth under anaerobic conditions was determined on LB medium by using the GasPak Anaerobic System (BBL) according to the manufacturer's instructions. Standard physiological tests were carried out according to the methods described by Cowan & Steel (1965) and Lányi (1987). Tests for

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NY-2^T is FJ158840.

A table giving the cellular fatty acid profiles of strain NY-2^T and the type strains of recognized *Jeotgalicoccus* species is available with the online version of this paper.

utilization of various substrates as sole carbon and energy sources were performed as described by Shirling & Gottlieb (1966); substrates were tested at a concentration of 0.1 % (w/v). Acid production from carbohydrates was determined as described by Leifson (1963). The chemotaxonomic characteristics of strain NY-2^T were determined by using cells cultured in LB medium. Analyses of cellular fatty acids, isoprenoid quinones and polar lipids were carried out by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. The DNA G+C content was determined by thermal denaturation (Marmur & Doty, 1962), and *Escherichia coli* K-12 was used as a standard. The phenotypic properties and biochemical characteristics of strain NY-2^T are summarized in Table 1,

Table 1. Differential phenotypic characteristics between strain NY-2^T and the type strains of recognized *Jeotgalicoccus* species

Strains: 1, NY-2^T; 2, *J. marinus* JSM 076033^T (data taken from Chen *et al.*, 2009); 3, *J. halotolerans* YKJ-101^T (unless indicated, data from Yoon *et al.*, 2003); 4, *J. psychrophilus* YKJ-115^T (Yoon *et al.*, 2003); 5, *J. pinnipedialis* A/G14/99/10^T (Hoyles *et al.*, 2004). +, Positive; w, weakly positive; –, negative; ND, no data available. All strains are positive for Gram stain, oxidase and catalase and negative for urease, nitrate reduction, decomposition of aesculin and acid production from lactose, melibiose, raffinose and trehalose.

Characteristic	1	2	3	4	5
Growth at/in:					
4 °C	–	–	+	+	–
37 °C	+	+	+	–	+
42 °C	+	+	+	–	+
45 °C	–	+	–	–	–
0 % (w/v) NaCl	+	–	+	–	–
14 % (w/v) NaCl	+	+	+	+	–
20 % (w/v) NaCl	+	+	+	–	–
23 % (w/v) NaCl	+	+	–	–	–
25 % (w/v) NaCl	–	+	–	–	–
Decomposition of gelatin	–	–	–	–	+
Acid production from:					
Cellobiose	–	+	–	–	–
D-Fructose	+	+	–	–	–
D-Galactose	–	+	–	–	ND
D-Glucose	–	+	–	–	–
D-Mannitol	–	+	+	–	–
D-Mannose	+	+	+	–	–
D-Xylose	+	–	–	–	–
L-Arabinose	+	+	+	–	–
Maltose	–	+	–	–	–
Predominant menaquinones (%)					
MK-6	0	39.3	13.5*	ND	5
MK-7	100	60.7	86*	80	89
DNA G+C content (mol%)	36.8	40.3	42	42	38.6

*Data from Chen *et al.* (2009).

together with those of the type strains of *J. marinus*, *J. halotolerans*, *J. psychrophilus* and *J. pinnipedialis*.

Genomic DNA extraction and PCR amplification of the 16S rRNA gene via universal primers were performed by using the method of Ausubel *et al.* (1999). The almost-complete 16S rRNA gene sequence of strain NY-2^T (1508 bp) was obtained, which was compared with those of the type strains of recognized *Jeotgalicoccus* species downloaded from the GenBank/EMBL database. Phylogenetic analysis was performed by using the software packages PHYLIP (Felsenstein, 1993) and MEGA version 3.1 (Kumar *et al.*, 2004) after multiple alignment of the data via CLUSTAL X (Thompson *et al.*, 1997). Distances (distance options according to Kimura's two-parameter model; Kimura, 1980, 1983) were calculated and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 500 resamplings (Felsenstein, 1985). Levels of DNA–DNA relatedness were determined according to the fluorometric microwell method (Ezaki *et al.*, 1989; He *et al.*, 2005).

The long-chain cellular fatty acids of strain NY-2^T were found to consist primarily of iso- and anteiso-methyl branched-chain components. The major fatty acids were iso-C_{15:0} (49.0 %) and anteiso-C_{15:0} (19.6 %). A comparison of the fatty acid profiles of strain NY-2^T and the type strains of recognized *Jeotgalicoccus* species is provided in Supplementary Table S1 (available in IJSEM Online). The predominant menaquinone of strain NY-2^T was MK-7 (100 %). The cellular polar lipids found in the novel strain were phosphatidylglycerol, diphosphatidylglycerol and several unidentified phospholipids. No aminolipids or glycolipids were detected. The G+C content of the DNA of strain NY-2^T was 36.8 mol%.

16S rRNA gene sequence comparisons revealed that strain NY-2^T was related most closely to *J. marinus* JSM 076033^T (99.9 % similarity), followed by *J. halotolerans* YKJ-101^T (96.2 %), *J. psychrophilus* YKJ-115^T (95.1 %), *J. pinnipedialis* A/G14/99/10^T (92.9 %), *Salinicoccus alkaliphilus* T8^T (91.5 %; Zhang *et al.*, 2002), *Salinicoccus hispanicus* DSM 5352^T (91.2 %; Ventosa *et al.*, 1992) and *Nosocomiicoccus ampullae* TRS-1^T (91.0 %; Alves *et al.*, 2008). Phylogenetic analysis based on 16S rRNA gene sequences thus revealed that strain NY-2^T was related most closely to the type strains of the four recognized species of the genus *Jeotgalicoccus* and that the novel isolate formed a distinct subclade with these four taxa in the phylogenetic trees that was supported by a high bootstrap resampling value (all three methods, 100 %) (Fig. 1). Species of the genera *Salinicoccus* and *Nosocomiicoccus* were the next nearest relatives of the novel strain, but they formed quite separate and robust clusters (Fig. 1). Signature nucleotides of species of the genus *Jeotgalicoccus* (Alves *et al.*, 2008) (GCUCC at position 86–90, GG at position 193–194, GU

Fig. 1. Phylogenetic dendrogram based on 16S rRNA gene sequence analysis constructed according to the neighbour-joining method showing the position of strain NY-2^T among its nearest neighbours. Statistically significant bootstrap values are shown at nodes and are expressed as percentages of 500 replications. Bar, 0.01 changes per nucleotide position.

at position 418–419 and GGC at position 1270–1273) were also found in the 16S rRNA gene sequence of strain NY-2^T.

The placement of strain NY-2^T within the genus *Jeotgalicoccus* was also confirmed based on phenotypic criteria, including chemical biomarkers. The major fatty acid profiles of strain NY-2^T and the type strains of *J. marinus*, *J. halotolerans*, *J. psychrophilus* and *J. pinnipedialis* were similar, primarily with iso-C_{15:0} and anteiso-C_{15:0} as the predominant components (Yoon *et al.*, 2003; Hoyles *et al.*, 2004; Chen *et al.*, 2009). The predominant menaquinone (MK-7), polar lipids (phosphatidylglycerol and diphosphatidylglycerol) and low DNA G+C content (36.8 mol%) of strain NY-2^T were also consistent with those of *Jeotgalicoccus* species (Yoon *et al.*, 2003; Hoyles *et al.*, 2004; Chen *et al.*, 2009). By contrast, strain NY-2^T could be distinguished from members of the genus *Salinicoccus*, which possess glycolipids, produce MK-6 as the major menaquinone and have a higher DNA G+C content (approximately 46–51 mol%) (Ventosa *et al.*, 1992). Strain NY-2^T could also be distinguished from members of the genus *Nosocomiicoccus*, whose major polar lipid is an unknown aminophospholipid and major fatty acids are C_{16:0} and C_{14:0} (Alves *et al.*, 2008).

DNA–DNA hybridization was employed to clarify further the relationship between the novel isolate and *J. marinus* JSM 076033^T. The level of DNA–DNA relatedness between strain NY-2^T and *J. marinus* JSM 076033^T was 28.8 %, well below the threshold value of 70 % recommended by Wayne *et al.* (1987) for assigning strains to different genomic species. It is therefore evident, based on phylogenetic analysis and DNA–DNA hybridization data, that strain NY-2^T represents a novel *Jeotgalicoccus* species.

Although strain NY-2^T shares many characteristics with *J. marinus*, it can be distinguished from *J. marinus* JSM 076033^T based on growth in the absence of NaCl and inability to grow at 45 °C or in the presence of 25 % NaCl (Chen *et al.*, 2009). *J. marinus* JSM 076033^T has significant amounts of MK-6 (39.3 %) whereas strain NY-2^T has only

MK-7 (100 %). The two taxa also differ regarding acid production from cellobiose, D-galactose, D-glucose, D-mannitol, D-xylose and maltose (Table 1). Therefore, on the basis of data from the present polyphasic study, we suggest that strain NY-2^T represents a novel species of the genus *Jeotgalicoccus*, for which the name *Jeotgalicoccus huakuii* sp. nov. is proposed.

Description of *Jeotgalicoccus huakuii* sp. nov.

Jeotgalicoccus huakuii (hua.ku'i.i. N.L. gen. n. *huakuii* of Hua-Kui, to honour Hua-Kui Chen, a respected Chinese microbiologist, for his enormous contributions to the development of microbiology in China).

Cells are Gram-stain-positive, non-spore-forming, non-motile cocci, approximately 0.4–0.8 µm in diameter. Colonies are white, circular, of low convexity and with clear margins, and approximately 0.5–1.0 mm in size after 2 days incubation on LB agar. Optimal growth occurs at 28–37 °C; no growth occurs below 4 °C or above 43 °C. Optimal pH for growth is 6.5–10.0; no growth is observed below pH 4.5. Growth occurs in the presence of 0–23 % (w/v) NaCl, with optimal growth at 3–8 % (w/v) NaCl. Growth occurs under both aerobic and anaerobic conditions. Catalase- and oxidase-positive. Urease-negative. Hydrolyses L-tyrosine but not aesculin, casein, hypoxanthine, starch, Tween 80, xanthine or gelatin. Nitrate is not reduced to nitrite. Acid is produced from L-arabinose, D-mannose, D-fructose and D-xylose, but not from cellobiose, D-glucose, lactose, maltose, melibiose, raffinose, trehalose, D-mannitol, sucrose, D-galactose or *myo*-inositol. MK-7 is the sole menaquinone. Major fatty acids (≥ 3 % of the total) are iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{17:0}, C_{16:0}, anteiso-C_{17:0} and iso-C_{16:0}. Major phospholipids are phosphatidylglycerol, diphosphatidylglycerol and several unidentified phospholipids. The DNA G+C content of the type strain is 36.8 mol%.

The type strain, NY-2^T (=CCTCC AB 208288^T =JCM 15687^T), was isolated from a seaside soil sample obtained from Shandong Province, China.

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