Jeotgalicoccus nanhaiensis sp. nov., isolated from intertidal sediment, and emended description of the genus *Jeotgalicoccus*

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A novel non-sporulating, non-motile, catalase- and oxidase-positive, strictly aerobic, Gram-positive-staining coccus, strain JSM 077023^T, was isolated from an intertidal sediment sample collected from Naozhou Island in the South China Sea, China. Growth occurred in the presence of 0.5–25 % (w/v) NaCl [optimum, 2–5 % (w/v) NaCl] and at pH 5.5–10.5 (optimum, pH 7.0–8.0) and at 4–45 °C (optimum, 30–35 °C). The major amino acid constituents of the cell wall were alanine, glycine and lysine. The major cellular fatty acids were anteiso-C_{15:0} and iso-C_{15:0}. The strain contained MK-7 and MK-6 as the predominant respiratory quinones and diphosphatidylglycerol, phosphatidylglycerol and an unidentified phospholipid as the polar lipids. The genomic DNA G + C content of strain JSM 077023^T was 41.3 mol%. A phylogenetic analysis based on 16S rRNA gene sequence comparisons revealed that strain JSM 077023^T should be assigned to the genus *Jeotgalicoccus* and was most closely related to the type strains of *Jeotgalicoccus halotolerans* (sequence similarity 99.0 %) and *Jeotgalicoccus aerolatus* (99.0 %), followed by *Jeotgalicoccus coquinae* (98.6 %) and *Jeotgalicoccus psychrophilus* (97.4 %). 16S rRNA gene sequence similarities of less than 97 % were observed with other species of the genus *Jeotgalicoccus*. Levels of DNA–DNA relatedness between strain JSM 077023^T and the type strains of *J. halotolerans*, *J. aerolatus*, *J. coquinae* and *J. psychrophilus* ranged from 36.8 to 22.7 %. The combination of phylogenetic analysis, DNA–DNA relatedness values, phenotypic characteristics and chemotaxonomic data supported the suggestion that strain JSM 077023^T represents a novel species of the genus *Jeotgalicoccus*, for which the name *Jeotgalicoccus nanhaiensis* sp. nov. is proposed. The type strain is JSM 077023^T (=DSM 23006^T=KCTC 13714^T). An emended description of the genus *Jeotgalicoccus* is also presented.

The genus *Jeotgalicoccus* was originally proposed by Yoon *et al.* (2003) with two species, *Jeotgalicoccus halotolerans* and *Jeotgalicoccus psychrophilus*. The genus was defined as comprising Gram-positive, catalase- and oxidase-positive, facultatively anaerobic, non-motile, non-sporulating cocci with MK-7 as the predominant isoprenoid quinone and a cell-wall peptidoglycan of the A3 α type [based on L-Lys–Gly_{3–4}–L-Ala (Gly)] (Yoon *et al.*, 2003). At the time of writing, the

genus comprised seven recognized species, the two named above plus *Jeotgalicoccus pinnipedialis* (Hoyle *et al.*, 2004), *Jeotgalicoccus marinus* (Chen *et al.*, 2009b), *Jeotgalicoccus huakuii* (Guo *et al.*, 2010), *Jeotgalicoccus aerolatus* (Martin *et al.*, 2011) and *Jeotgalicoccus coquinae* (Martin *et al.*, 2011). During an investigation of the microbial diversity of Naozhou Island in the South China Sea (Chen *et al.*, 2009a, b), a strictly aerobic, Gram-positive-staining coccus, strain JSM 077023^T, was isolated from an intertidal sediment sample. Based on the results of a study using a polyphasic taxonomic approach, this strain is considered to represent a novel species of the genus *Jeotgalicoccus*.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JSM 077023^T is FJ237390.

A supplementary figure is available with the online version of this paper.

Strain JSM 077023^T was isolated from an intertidal sediment sample by plating 1:10 serial dilutions of the sample on marine agar 2216 (MA, pH 7.5; Difco) at 28 °C for 14 days. After primary isolation, the strain was purified by repeated streaking and subculturing on MA plates (4–5 times) and examined by light microscopy. The isolate was preserved both on MA slants at 4 °C and as 20 % (v/v) glycerol stocks at –80 °C. For comparison, four reference strains, *J. halotolerans* DSM 17274^T, *J. aerolatus* DSM 22420^T, *J. coquinae* DSM 22419^T and *J. psychrophilus* DSM 19085^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). Unless indicated otherwise, morphological, physiological, molecular and chemotaxonomic studies were performed with cells grown on MA (pH 7.5) at 30 °C.

Cell morphology was examined by using light microscopy (DM3000; Leica). Gram staining and the KOH lysis test were carried out according to Smibert & Krieg (1994) and Gregersen (1978), respectively. Growth was tested at various temperatures (4, 5–55 °C, in increments of 5 °C) and at different pH values (5.0–11.0, in increments of 0.5 pH units) on MA as well as in marine broth 2216 (MB, pH 7.5; Difco). Growth in the absence of NaCl was investigated on nutrient agar (NA) prepared according to the formula of Atlas & Parks (1993) except that NaCl was excluded. Tolerance of NaCl was tested on NA at different NaCl concentrations [0.1 and 0.5 % (w/v) and 1–30 % (w/v) in increments of 1 %]. Growth under anaerobic conditions was determined on MA and NA supplemented with 0.5 % (w/v) glucose and with or without 0.1 % (w/v) nitrate by using the GasPak Anaerobic Systems (BBL) according to the manufacturer's instructions. Urease activity and decomposition of casein, DNA, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80 and xanthine were determined as described by Cowan & Steel (1965). Methyl red and Voges–Proskauer tests and the determination of hydrolysis of aesculin, indole and H₂S production, nitrate reduction, and activities of lysine decarboxylase, phenylalanine deaminase and ornithine decarboxylase were assessed as recommended by Smibert & Krieg (1994). Tests for acid production from carbohydrates and utilization of carbon and nitrogen sources were performed as recommended by Ventosa *et al.* (1982). Observation of motility and tests for catalase and oxidase activities were determined as described previously (Chen *et al.*, 2007). Other enzymic activities were also assayed by using API ZYM strips (bioMérieux) according to the manufacturer's instructions.

Strain JSM 077023^T was strictly aerobic and the cells were Gram-positive-staining, non-motile and non-sporulating cocci. Colonies were creamy white, circular, somewhat convex, non-translucent and 1–2 mm in diameter after incubation at 30 °C for 3–4 days on MA. The strain grew optimally in presence of 2–5 % (w/v) NaCl, at 30–35 °C and at pH 7.0–8.0. Detailed phenotypic properties that differentiated strain JSM 077023^T from recognized species of the

genus *Jeotgalicoccus* are summarized in Table 1 and also detailed in the species description below.

DNA was isolated according to the method of Hopwood *et al.* (1985) and the G + C content was determined by using the HPLC method (Mesbah *et al.*, 1989). The 16S rRNA gene sequence was amplified by PCR and sequenced as described by Cui *et al.* (2001). Pairwise sequence similarity was calculated by using a global alignment algorithm, implemented at the EzTaxon server (Chun *et al.*, 2007). Phylogenetic analysis was performed by using the MEGA version 4.1 software package (Tamura *et al.*, 2007) after multiple alignment of the sequence data with CLUSTAL_X (Thompson *et al.*, 1997). Distances were calculated by using distance options according to Kimura's two-parameter model (Kimura, 1980) and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). Maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) trees were generated by using the tree-making algorithms contained in the PHYLIP package (Felsenstein, 2002). Confidence values for the branches of phylogenetic trees were determined by using bootstrap analyses (based on 1000 resamplings) (Felsenstein, 1985). DNA–DNA hybridization experiments were performed using the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992).

The DNA G + C content of strain JSM 077023^T was 41.3 mol%. An almost-complete 16S rRNA gene sequence (1455 bp) was determined. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain JSM 077023^T should be assigned to the genus *Jeotgalicoccus*. The novel strain was most-closely related most to the type strains of *J. halotolerans* (99.0 % 16S rRNA gene sequence similarity), *J. aerolatus* (99.0 %), *J. coquinae* (98.6 %) and *J. psychrophilus* (97.4 %). Less than 97 % 16S rRNA gene sequence similarity was observed with other species of the genus *Jeotgalicoccus*: *J. marinus* (96.1 %), *J. huakuii* (96.1 %) and *J. pinnipedialis* (93.3 %). The neighbour-joining phylogenetic tree further confirmed that strain JSM 077023^T was phylogenetically closely related to species of the genus *Jeotgalicoccus* and formed an independent subcluster with *J. halotolerans* YKJ-101^T and *J. aerolatus* MPA-33^T (Fig. 1). However, strain JSM 077023^T occupied a distinct lineage in the phylogenetic trees constructed by using the maximum-likelihood and maximum-parsimony methods (see Supplementary Fig. S1, available in IJSEM Online). Levels of DNA–DNA relatedness between strain JSM 077023^T and the type strains of *J. halotolerans*, *J. aerolatus*, *J. coquinae* and *J. psychrophilus* were 36.8, 35.4, 30.5 and 22.7 %, respectively, values that were well below the threshold (70 %) recommended by Wayne *et al.* (1987) for the definition of members of a species. Therefore, it would appear that, on the basis of the phylogenetic and DNA–DNA hybridization data, strain JSM 077023^T represents a novel species of the genus *Jeotgalicoccus* according to accepted criteria (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994).

Table 1. Characteristics that differentiate strain JSM 077023^T from phylogenetically related species of the genus *Jeotgalicoccus*

Taxa: 1, strain JSM 077023^T; 2, *J. halotolerans* DSM 17274^T; 3, *J. aerolatus* DSM 22420^T; 4, *J. coquinae* DSM 22419^T; 5, *J. psychrophilus* DSM 19085^T. +, Positive reaction; –, negative reaction; ND, no data available. All strains are non-motile, non-sporulating, catalase- and oxidase-positive, Gram-positive cocci. All strains are negative in tests for urease activity, nitrate reduction, H₂S and indole production and decomposition of aesculin, casein, DNA, hypoxanthine, Tweens 40, 60, 80 and xanthine. Negative result in methyl red and Voges–Proskauer tests. Negative result in tests for acid production from adonitol, cellobiose, D-galactose, myo-inositol, lactose, melezitose, melibiose, raffinose and D-sorbitol.

Characteristic	1	2	3	4	5
Colony pigmentation*	CW	LY	CW	CW	LY
Facultatively anaerobic	–	+	–	–	+
Growth in NaCl (w/v):					
0 %	–	+	+	+	+
20 %	+	+	–	–	–
25 %	+	–	–	–	–
Temperature range (°C)	4–45	4–40	10–40	10–40	4–35
Temperature optimum (°C)	30–35	30–35	25–30	25–30	20–25
pH range	5.5–10.5	6–10	6–10	6–10.5	6.0–10.5
pH optimum	7.0–8.0	7.0–8.0	7.0–8.0	7.5–8.0	7.5–8.5
Decomposition of:					
Gelatin	–	–	–	+	–
Starch	–	–	–	+	–
Tween 20	–	–	–	+	+
Acid production from:					
L-Arabinose	–	+	–	–	–
D-Fructose	+	–	+	–	–
D-Glucose	+	+	–	–	+
Glycerol	+	–	–	–	–
Maltose	+	–	–	–	+
D-Mannitol	+	+	–	+	–
D-Mannose	+	+	+	+	–
L-Rhamnose	+	+	+	+	–
D-Ribose	+	+	–	–	–
Sucrose	+	–	–	–	+
Trehalose	+	–	–	–	–
D-Xylose	+	–	–	+	–
Menaquinones (%)†					
MK-6	11.3	13.5	24.0	49.8	ND
MK-7	85.8	82.1	75.4	50.2	80
MK-8	2.9	4.5	0.6	0	ND

*CW, creamy white; LY, light yellow.

†Data for the type strains of *J. halotolerans* and *J. psychrophilus* were taken from Chen *et al.* (2009b) and Yoon *et al.* (2003), respectively; those for the type strains of *J. aerolatus* and *J. coquinae* were from Martin *et al.* (2011). Other data were from this study.

Isolation of the cell-wall fraction and preparation of cell-wall hydrolysates were carried out by using the method of Schleifer (1985). The amino acid composition of the cell-wall hydrolysates was detected by using HPLC, as described by Tang *et al.* (2009). Polar lipids were extracted according to the method of Minnikin *et al.* (1979) and were identified by two-dimensional TLC and spraying with the appropriate detection reagents (Collins & Jones, 1980). Isoprenoid quinones were isolated by using the methods of Minnikin *et al.* (1984) and were then separated by HPLC (Kroppenstedt, 1982, 1985). Fatty acid compositions were determined according to Sasser (1990) by using the Microbial Identification system (Microbial ID) with cells

grown in MB in flasks on a rotary shaker (with shaking at 200 r.p.m.) at 30 °C for 2 days.

The chemotaxonomic data obtained for strain JSM 077023^T were consistent with the assignment of the strain to the genus *Jeotgalicoccus* (Yoon *et al.*, 2003). The major amino acid constituents of the cell-wall hydrolysates were alanine, glycine and lysine, which corresponded to the A3 α peptidoglycan type, based on L-Lys–Gly_{3–4}–L-Ala (Gly), as previously described for the genus *Jeotgalicoccus*. The fatty acid profile of strain JSM 077023^T was similar to those of the type strains of the four phylogenetically related species of the genus *Jeotgalicoccus*, although there were differences

Fig. 1. Phylogenetic tree showing the position of strain JSM 077023^T among related taxa based on 16S rRNA gene sequences constructed by using the neighbour-joining method. Numbers at nodes are bootstrap percentages (>50%) based on a neighbour-joining analysis of 1000 resampled datasets. Bar, 2 substitutions per 100 nt.

in the proportions of some components (Table 2). The major fatty acids (>10% of the total) of strain JSM 077023^T were anteiso-C_{15:0} (45.6%) and iso-C_{15:0} (28.0%). Strain JSM 077023^T contained MK-7 (85.8%) and MK-6 (11.3%) as the predominant respiratory quinones with MK-8 (2.9%) present in trace amounts (Table 1). The polar lipids of strain JSM 077023^T consisted of diphosphatidylglycerol, phosphatidylglycerol and an unidentified phospholipid.

The results of the phylogenetic analysis and of the morphological and chemotaxonomic investigations supported the affiliation of strain JSM 077023^T to the genus *Jeotgalicoccus* (Yoon *et al.*, 2003). However, the novel strain differed markedly from the seven recognized species of the genus *Jeotgalicoccus* by its ability to tolerate up to 25% (w/v) NaCl and temperatures of 45 °C, by its ability to produce acid from glycerol and trehalose. The novel strain was unable to grow in the absence of NaCl and also differed from the other members of the genus *Jeotgalicoccus* in some chemotaxonomic characteristics (Tables 1 and 2). In conclusion, phylogenetic analysis based on 16S rRNA gene sequences, the DNA–DNA relatedness results and the phenotypic and chemotaxonomic data presented here support the proposal of strain JSM 077023^T as representing a novel species of the genus *Jeotgalicoccus*, for which the name *Jeotgalicoccus nanhaiensis* sp. nov. is proposed.

Description of *Jeotgalicoccus nanhaiensis* sp. nov.

Jeotgalicoccus nanhaiensis (nan.hai.en'sis. N.L. masc. adj. *nanhaiensis* pertaining to Nanhai, the Chinese name for the

South China Sea, the source of the sediment sample from which the type strain was isolated).

Cells are Gram-positive-staining, non-motile, non-sporulating, strictly aerobic, catalase- and oxidase-positive, coccus-shaped, 0.7–1.1 µm in diameter and occur singly or in pairs, tetrads or clumps. Colonies are creamy white, circular, somewhat convex, non-translucent, have glistening surfaces and entire margins and are 1–2 mm in diameter on marine agar 2216 (Difco). No diffusible pigments are produced. Growth occurs in the presence of 0.5–25% (w/v) NaCl (optimally at 2–5%) and at 4–45 °C (optimally at 30–35 °C) and at pH 5.5–10.5 (optimally at pH 7.0–8.0). Negative result in methyl red and Voges–Proskauer tests. Negative results in tests for the decomposition of aesculin, casein, DNA, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80 and xanthine. Nitrate is not reduced to nitrite. H₂S and indole are not produced. The following compounds are utilized as sole sources of carbon and energy: acetic acid, arbutin, glycerol and *myo*-inositol. The following compounds are not utilized: L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, glycogen, lactose, maltose, D-mannose, melezitose, melibiose, raffinose, L-rhamnose, D-ribose, sucrose, trehalose, D-xylose, adonitol, D-mannitol, D-sorbitol, salicin, butyrate, citrate, formate, fumarate, gluconate, malate, malonate, propionate, succinate, L-alanine, L-arginine, L-asparagine, L-glutamic acid, L-glycine, L-histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-methionine, L-proline, L-serine and L-valine. Produces acid from D-fructose, D-glucose, maltose, D-mannose, L-rhamnose, D-ribose, sucrose, trehalose,

Table 2. Fatty acid compositions of strain JSM 077023^T and related species of the genus *Jeotgalicoccus*

Strains: 1, JSM 077023^T; 2, *J. halotolerans* DSM 17274^T; 3, *J. aerolatus* DSM 22420^T; 4, *J. coquinae* DSM 22419^T; 5, *J. psychrophilus* DSM 19085^T. Data are percentages of the total fatty acid content. –, Not detected. All data were from this study.

Fatty acid	1	2	3	4	5
Saturated:					
C _{10:0}	–	–	0.1	0.1	–
C _{14:0}	0.8	0.7	2.3	0.5	1.1
C _{16:0}	2.5	3.5	8.8	4.3	3.8
C _{18:0}	0.3	–	2.5	0.7	0.4
C _{20:0}	0.1	–	–	0.1	0.1
Unsaturated:					
C _{16:1} ω7c alcohol	0.8	0.7	–	0.6	1.1
C _{16:1} ω11c	0.3	0.6	1.2	0.4	0.5
Branched:					
iso-C _{13:0}	0.2	–	0.2	–	0.1
anteiso-C _{13:0}	0.1	–	–	–	0.1
iso-C _{14:0}	2.6	3.6	4.8	3.3	6.5
iso-C _{15:0}	28.0	22.5	30.6	22.5	26.0
anteiso-C _{15:0}	45.6	38.1	35.8	42.6	42.1
iso-C _{15:1} ω9c	0.2	0.1	–	0.2	–
iso-C _{16:0}	6.3	12.3	4.6	8.4	8.1
iso-C _{17:0}	3.2	4.7	3.9	7.0	4.5
anteiso-C _{17:0}	4.7	8.8	1.7	5.3	2.9
C _{17:0} 3-OH	0.1	0.1	–	–	0.1
iso-C _{17:1} ω10c	1.9	2.5	–	1.0	1.0
iso-C _{18:0}	–	0.5	–	0.3	0.1
C _{18:1} ω9c	0.7	–	0.8	0.7	0.3
iso-C _{19:0}	0.4	0.6	1.0	1.2	0.4
anteiso-C _{19:0}	0.2	–	–	0.4	–
Summed features*					
2	0.1	–	0.5	–	–
3	0.4	0.5	1.0	0.3	0.2
4	0.4	–	–	–	0.1

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 2 comprises iso-C_{16:1} I and/or C_{14:0} 3-OH. Summed feature 3 comprises C_{16:1}ω7c and/or C_{16:1}ω6c. Summed feature 4 comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B.

D-xylose, glycerol and D-mannitol, but not from N-acetylglucosamine, L-arabinose, cellobiose, D-galactose, lactose, melezitose, melibiose, raffinose, starch, adonitol, dulcitol, myo-inositol, D-salicin, D-sorbitol and xylitol. Constitutive enzymes expressed are N-acetyl-β-glucosaminidase, cystine arylamidase, α-fucosidase, α- and β-galactosidase, α- and β-glucosidase, β-glucuronidase, lipase (C14) and trypsin. Acid phosphatase, alkaline phosphatase, α-chymotrypsin, esterase (C4), esterase lipase (C8), leucine arylamidase, lysine decarboxylase, α-mannosidase, naphthol-AS-BI-phosphohydrolase, ornithine decarboxylase, phenylalanine deaminase, valine arylamidase and urease are not observed. The major amino acid constituents of the cell wall are alanine, glycine and lysine.

Possesses MK-7 and MK-6 as the predominant menaquinones. Polar lipids comprise diphosphatidylglycerol, phosphatidylglycerol and an unidentified phospholipid. The major cellular fatty acids are anteiso-C_{15:0} and iso-C_{15:0}.

The type strain, JSM 077023^T (=DSM 23006^T=KCTC 13714^T), was isolated from an intertidal sediment sample collected from Naozhou Island in the South China Sea, China. The DNA G+C content of the type strain is 41.3 mol% (HPLC method).

Emended description of the genus *Jeotgalicoccus* Yoon *et al.* 2003

The description is as given by Yoon *et al.* (2003) with the following changes. Aerobiosis or facultative anaerobiosis. Predominant menaquinones are MK-7 and MK-6. The genomic DNA G+C content is 36–42 mol%.

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