**Sinomicrobium pectinilyticum** sp. nov., a pectinase-producing bacterium isolated from alkaline and saline soil, and emended description of the genus *Sinomicrobium*

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A Gram-reaction-negative, non-spore-forming strain, designated 5DNS001^T, was isolated from soil of an ancient salt-extracting facility in China. Analysis of the almost-complete 16S rRNA gene sequence of the bacterium suggested that it belongs to the genus *Sinomicrobium* in the family *Flavobacteriaceae*. It exhibited highest 16S rRNA gene sequence similarity with *Sinomicrobium oceani* SCSIO 03483^T* (96.3 %), but less than 93 % sequence similarity with members of the genera *Imtechella*, *Zhouia* and *Joostella* and other recognized members of the family *Flavobacteriaceae*. The strain was able to hydrolyse pectin and starch by producing pectinase and \( \alpha \)-amylase. The DNA G+C content of the strain was 42.6 mol%. The major respiratory quinone was MK-6. The major polar lipid detected in the strain was phosphatidylethanolamine. The dominant cellular fatty acids were iso-C\(_{15}:0\), iso-C\(_{17}:0\) 3-OH and summed feature 3 (C\(_{16:1}\)ω6c/ C\(_{16:1}\)ω7c). Based on phenotypic, genotypic, chemotaxonomic and phylogenetic analyses, a novel species, *Sinomicrobium pectinilyticum*, is proposed. The type strain is 5DNS001^T (=CGMCC1.11000^T=KCTC23776^T).

The genus *Sinomicrobium* was proposed by Xu et al. (2013) to include Gram-negative, aerobic, rod-shaped, non-spore-forming and motile (by gliding) marine bacteria that possess MK-6 as a major respiratory quinone and phosphatidylethanolamine as a main polar lipid. The DNA G+C content of the type strain, SCSIO 03483^T*, of a single species of the genus, *Sinomicrobium oceani*, isolated from an abyssal sediment sample of the South China Sea, was 38.4 mol% (Xu et al., 2013). In addition to this species, many members of the family *Flavobacteriaceae* have been isolated from marine environments, such as seawater, sediments, sponge and tidal flat sediment. In addition, strains have been isolated from saline or alkaline soils (Ahmed et al., 2007; Chen et al., 2008). However, the descriptions of novel taxa of the family *Flavobacteriaceae* isolated from alkaline and saline soils are rare. During recent studies of the microbiological diversity of an ancient (Shang or Zhou Dynasty) salt-extracting facility, a novel aerobic, yellow-pigmented bacterial strain, designated 5DNS001^T, was isolated. In this study, based on its distinctiveness from the reference strain *S. oceani* SCSIO 03483^T*, the strain is suggested to represent a novel species of the genus *Sinomicrobium*.

A soil sample was collected from the ruins of an ancient salt-extracting facility in Dongying city (Shandong Province, China), located at the mouth of the Yellow River (37° 14′ 50″ N 118° 41′ 11″ E). At this facility, salt was extracted from seawater using simple technology around 206 BC. The salinity of the soil sample was 0.6 % and pH was 9.3. The strain was isolated by the plating method using the following enrichment medium (per litre): 20 g MgCl\(_2\), 6H\(_2\)\( \text{O}\), 5 g K\(_2\)SO\(_4\), 0.1 g CaCl\(_2\), 0.1 g yeast extract, 0.5 g NH\(_4\)Cl, 0.05 g KH\(_2\)PO\(_4\), 50 g NaCl, 0.2 g tryptone, 0.5 g casein and 2 g citrate sodium. pH was adjusted to 9.0 with phosphate buffer. After incubation at 25 °C for 1 week, strain 5DNS001^T was isolated by plating the enrichment on the same medium. The strain was stored as freeze-dried powder in skimmed milk or 20 % (v/v) glycerol in artificial seawater at −80 °C for long-term preservation. For routine cultivation and maintenance, marine agar 2216 (MA; Difco) was used except where indicated. *S. oceani* SCSIO 03483^T* was used as a reference strain and cultured under the same conditions.
After cultivation at 25 °C in MA, the genomic DNA of the strain was isolated with a DNA extraction kit (Promega). The 16S rRNA gene was amplified by using the set of universal primers 27F and 1492R according to the procedure described by Brosius et al. (1978). The PCR product was purified with a Qiagen II Extraction kit, ligated into the pGM-18T vector (Promega) and transformed into Escherichia coli DH5α competent cells. The recombinant DNA was sequenced with an ABI 3700 capillary sequencer (PE Applied Biosystems). An almost-complete 16S rRNA gene fragment (1480 bp) was determined. After multiple alignments of the obtained 16S rRNA gene sequence by using CLUSTAL X (Thompson et al., 1997), phylogenetic trees were reconstructed using MEGA version 5.0, and clustered with the neighbour-joining and maximum-likelihood methods (Tamura et al., 2011). Evolutionary distances were calculated using the Jukes & Cantor (1969) model. Bootstrap analysis (based on 1000 resamplings) was used to evaluate the tree topology of the neighbour-joining data (Felsenstein, 1985). An unrooted tree reconstructed using the neighbour-joining method showed the phylogenetic position of strain 5DNS001T within the family Flavobacteriaceae (Fig. 1). Strain 5DNS001T was a member of the genus Sinomicrobium and formed a branch with S. oceani SCSIO 03483T. It exhibited highest 16S rRNA gene sequence similarity with S. oceani SCSIO 03483T (96.3 %), and less than 93 % similarity with members of the genera Imetechella, Sediminicola, Zhouia, Leeuwenhoekiella, Lutaemella, Joostella and Flavobacterium and other recognized members of the family Flavobacteriaceae. The topology of the tree generated with the maximum-likelihood method was similar (data not shown).

Cell morphology of strain 5DNS001T was examined by scanning electron microscopy (SEM 200; FEI Quanta), using exponential phase cells incubated in marine broth (MB). Growth under anaerobic conditions was assessed using the Hungate anaerobic technique. Gliding motility was observed according to the hanging drop method (Perry, 1973) under a ×1000 oil immersion objective, using low concentration MA (50 %). Using the method described by Dong & Cai (2001), Gram staining was determined using cells grown in MB at 25 °C for 24 h. Endospore formation and catalase activity were tested according to the method described by Dong & Cai (2001). Oxidase activity was determined using an oxidase reagent (REF55635; bioMerieux). The production of flexirubin-type pigments was investigated using the KOH test, following the minimal standards for the description of new taxa in the family Flavobacteriaceae (Bernardet et al., 2002). After 2 days of growth at 25 °C, colonies grown on MA plates were yellow and translucent. Cells were Gram-reaction-negative, aerobic, non-spore-forming, motile by means of gliding and rod-shaped (0.3–0.5 μm wide and 3.5–4.2 μm long) (Fig. S1, available in the online Supplementary Material). Flexirubin-type pigments were not produced.

The requirement for and tolerance to NaCl were determined at 25 °C with 0–12 % (w/v) NaCl in increments of 1 % in Luria–Bertani (LB) medium. The pH range for growth was investigated at pH 4.0–10.0 (at intervals of 1 pH unit) using MB adjusted with the following buffers (0.1 M): citric acid/ sodium citrate for pH 3.0–5.0, KH2PO4/NaOH for pH 6.0–8.0 and NaHCO3/Na2CO3 for pH 9.0–10.0. pH was verified after autoclaving. The temperature range for growth was

![Fig. 1. Neighbour-joining tree showing the phylogenetic relationships between strain 5DNS001T and its closest relatives inferred from 16S rRNA gene sequences. Bootstrap percentages (based on 1000 replications) are shown at branch points; only values ≥60 % are shown. Bar, 0.01 substitutions per nucleotide position.](image-url)
measured at 4, 10, 15, 20, 25, 30, 37, 40, 45 and 50 °C in MB. In all of these growth tests, S. oceani SCSIO 03483T was used as a reference and tested at the same conditions. After 7 days of cultivation, growth was observed at 10–45 °C (optimum, 25 °C), at pH 6.0–9.0 (optimum, pH 8.0) and with 0–9 % NaCl (optimum, 0–3 %). For S. oceani SCSIO 03483T, almost similar growth conditions were observed in the same medium, but with minor differences, including pH range of 6.0–9.0 (optimum, pH 7.0) and NaCl tolerance of 0–10 % (optimum, 0–4 %).

Hydrolysis of casein and starch was tested on MA supplemented with 1 % (w/v) casein or 0.5 % (w/v) soluble starch, according to the method described by Dong & Cai (2001). Acid production from carbohydrates was determined using the methods described by Gordon et al. (1974). Other biochemical tests were carried out at 25 °C with API 20E, API 20E and API ZYM strips (bioMérieux) according to the manufacturer’s instructions, and with the Biolog GN2 MicroPlate system. These tests were done using S. oceani SCSIO 03483T as a reference strain. The main physiological, biochemical and morphological characteristics of strain 5DNS001T are given in Table 1 and in the species description. In contrast to S. oceani SCSIO 03483T, strain 5DNS001T was able to hydrolyse pectin and starch in MA and produce novel pectinase and α-amylase (Li et al., 2014).

For fatty acid analysis, cells of strain 5DNS001T and S. oceani SCSIO 03483T grown at 25 °C on MA plates were extracted, saponified and esterified; this was followed by GC analysis of the fatty acid methyl esters using the standard protocol of version 6.0 of the Sherlock MIDI system (Sasser, 1990). Profiles were compared with the MIDI identification database TSBA6.0 (MIDI). Respiratory quinones were purified by TLC and the extract was analysed using reversed-phase HPLC on a C18 column eluted with methanol/2-propanol (2 : 1 by volume) at 1 ml min−1 (Komagata & Suzuki, 1987). Using reversed-phase HPLC, the DNA G+C contents of strain 5DNS001T and the reference strain were determined according to the method described by Mesbah & Whitman (1989), using the DNA of E. coli DH5 as a standard. Cellular polar lipids were extracted and separated by two-dimensional TLC, using aluminium-backed silica gel plates (Merck), as described by Tindall (1990). As shown in Table S1, the major fatty acids in strain 5DNS001T were (making up 51 % of the total) iso-C15:0 (24.8 %), iso-C17:0 3-OH (9.4 %) and summed feature 3 (comprising C16:1ω6c/C16:1ω7c, 16.8 %). There were minor composition differences between the two strains. The fatty acid iso-C15:1F (2.4 %) was detected only in strain 5DNS001T, whereas iso-C15:0 3-OH (2.7 %) and C16:0 3-OH (2.3 %) were observed only in S. oceani SCSIO 03483T (Table S1). The major respiratory quinone of strain 5DNS001T was menaquinone MK-6, which is in line with that found in the reference strain. The DNA G+C content of strain 5DNS001T was 42.6 mol%, Phosphatidylethanolamine (PE) was detected as the predominant polar lipid in strain 5DNS001T, as well as in the reference strain (Xu et al., 2013) (Fig. S2). In addition, two unidentified phospholipids (PL1 and PL2) and one unidentified amino lipid (AL2) were detected in strain 5DNS001T, whereas only two unidentified amino lipids (AL1 and AL2) were found in the reference strain.

On the basis of the results of 16S rRNA gene sequence comparison, DNA G+C content, fatty acid, and other morphological, physiological and chemotaxonomic characteristics listed above, strain 5DNS001T is considered to represent a novel species of the genus Sinomicrobium, for which the name Sinomicrobium pectinilyticum sp. nov. is proposed.

Emended description of the genus Sinomicrobium Xu et al. 2013

The description is as given by Xu et al. (2013) with the following amendments. Catalase and oxidase activities are variable among species. The major polar lipid is phosphatidylethanolamine, together with unidentified phospholipids or an unidentified amino lipid.
Description of Sinomicrobium pectinilyticum sp. nov.

Sinomicrobium pectinilyticum [pec.tin.i.ly’ti.cum. N.L. n. pectinum pectin; N.L. adj. lyticus -a -um (from Gr. adj. lutiko -a -on) able to loosen, able to dissolve; N.L. neut. adj. pectinilyticum pectin-dissolving].

Cells are non-spore-forming and strictly aerobic with gliding motility. Cells are 0.3–0.5 μm in width and 3.5–4.2 μm in length in MA; lack flagella. Colonies on MA plates are circular, slightly raised, smooth and yellow. Produces diffusible yellow pigments. Positive for oxidase, β-glucosidase (asculin hydrolysis), gelatin, casein, starch and pectin hydrolysis, but negative for catalase, nitrate reduction, indole production, D-glucose fermentation, and pectin hydrolysis, but negative for catalase, nitrate reduction, indole production, D-glucose fermentation, and pectin hydrolysis. Growth occurs at 10–45 °C, with no growth on glucose, sucrose, melibiose and arabinose in the API 20E system are seen for gelatinase, acetoin production, and glucose, sucrose, melibiose and arabinose.

The type strain, 5DNS001 T (CGMCC1.11000 T = KCTC23776 T), was isolated from a saline and alkaline soil of an ancient salt-extracting facility in Dongying city, China. The DNA G+C content of the type strain is 42.6 mol%.

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References


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