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Short Communication

Sporomusa intestinalis sp. nov., a homoacetogenic bacterium isolated from the gut of a higher termite, *Termes comis* (Termitinae)

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The genus *Sporomusa* belongs phylogenetically to the family *Vellionellaceae* of the phylum *Firmicutes*. The members of this genus are known to be anaerobic or aerotolerant, homoacetogenic bacteria which grow autotrophically on H_2/CO_2 or heterotrophically on various substrates such as sugars, alcohols, amino acids, and organic acids. To date, the genus *Sporomusa* comprises nine species: *Sporomusa ovata*, *Sporomusa sphaeroides*, *Sporomusa acidovorans*, *Sporomusa paucivorans*, *Sporomusa termitida*, *Sporomusa malonica*, *Sporomusa silvacetica*, *Sporomusa aerivorans*, and *Sporomusa rhizae* (Boga et al., 2003; Breznak et al., 1988; Dehning et al., 1989; Gößner et al., 2006; Hermann et al., 1987; Kuhner et al., 1997; Möller et al., 1984; Ollivier et al., 1985). Of these *Sporomusa* species, *S. termitida* and *S aerivorans* have been isolated from wood-feeding and soil-feeding higher termites, respectively. In this paper, we describe the isolation and characterization of a novel *Sporomusa* species, designated strain Tc2A^T (JCM13218^T=DSM17189^T) from the gut content of wood/soil interface-feeding higher termite, and propose a new species to accommodate it.

Strain Tc2A^T was obtained from the gut of the interface-feeding higher termite *Termes comis* (Termitinae), which was collected in Pathum Thani, Thailand. For enrichment of strain Tc2A^T, the guts were dissected and homogenized, followed by inoculation into 20 ml of a strictly anaerobic bicarbonate medium (Hattori et al., 2000) supplemented with 2 mM dithiothreitol, 200 ppm Bacto yeast extract, and 40 mM bromoethane sulfonate, 2 mM sodium acetate and H_2/CO_2 (100 kPa, 80 : 20, v/v). After 3 weeks' incubation at pH 7 and 30°C, the enrichment culture was serially diluted into the fresh medium (inoculum 10%, v/v). After the repeated transfers of the enrichment culture, they were further inoculated into the fresh medium solidified with 2% agar. It was incubated at 30°C for 2 weeks to form colonies. Isolation of strain Tc2A^T was achieved by 5 successive transfers of the colony into the agar medium. The isolate was examined for physiological and biochemical characteristics. Utilization of electron donors and acceptors, ef-

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fects of temperature, pH, NaCl, and KCl on growth were determined according to the method of Hattori et al. (2000). All the measurements were carried out at least in duplicate. The growth was evaluated by measuring OD_{600}. Both inoculated medium without substrate and uninoculated medium with substrate were used as negative controls. Substrate utilization and product formation were analyzed by HPLC (Agilent model 1100) equipped with a UV and a refractive index detector. Sulfate and nitrate were measured by ion chromatograph as described previously (Sato et al., 2009). Catalase was tested as described previously (Smibert and Krieg, 1994). Gram stain was performed with a commercially available kit (Favor-G kit; Nissui Pharmaceutical, Tokyo, Japan). KOH reaction to determine Gram reaction was carried out according to the method of Buck (1982). Extraction and isolation of DNA from strain Tc2A^T were performed as described elsewhere (Ohkuma and Kudo, 1996). DNA base composition analysis and DNA-DNA hybridization were carried out as described previously (Itoh et al., 1998). The type strain of *Sporomusa termitida* (DSM4440) was used as a reference for the hybridization experiments. For a phylogenetic analysis of the strain Tc2A^T, the partial 16S rRNA gene was amplified using primers 27F and 1492R (Hongoh et al., 2003). The PCR amplicons were ligated into a pGEM-T vector (Promega, Tokyo, Japan) and introduced into *Escherichia coli*. The cloned sequences were amplified by PCR with M13 forward and M4 reverse primer set, and the sequences of the PCR products were determined using the following primers: SP6, T7, and 533F (Hongoh et al., 2003). The obtained sequence was incorporated into a modified ARB database (Ludwig et al., 2004; Sato et al., 2009). A phylogenetic tree was constructed by using a maximum-likelihood method in the MEGA program version 5.1 (Tamura et al., 2011).

Strain Tc2A^T was an anaerobic, spore-forming slightly curved rod-shaped bacterium that could grow autotrophically or heterotrophically on various substrates as listed in the species description (Fig. 1). The main product from growth-supportive substrate was acetate, indicating that the strain was an acetogen. Phylogenetic analysis of strain Tc2A^T based on 16S rRNA gene sequencing revealed that the strain was affiliated to the genus *Sporomusa* (Fig. 2). The closest relative of strain Tc2A^T was *S. termitida,* with a high bootstrap confidence value (100%). However, the sequence similarity between strain Tc2A^T and *S. termitida* was relatively low (96.0% similarity). In addition, the results from the DNA-DNA hybridization study showed that the degree of DNA relatedness between strain Tc2A^T and *S. termitida* was only 17.1%. These results strongly indicated that the strain Tc2A^T was separated from the related species of the genus *Sporomusa*. The differential physiological and biochemical characteristics of strain Tc2A^T and related species are summarized in Table 1. Among the *Sporomusa* species, strain Tc2A^T and the two species from termites (*S. termitida* and *S. aerivorans*) shared an ability to utilize mannitol

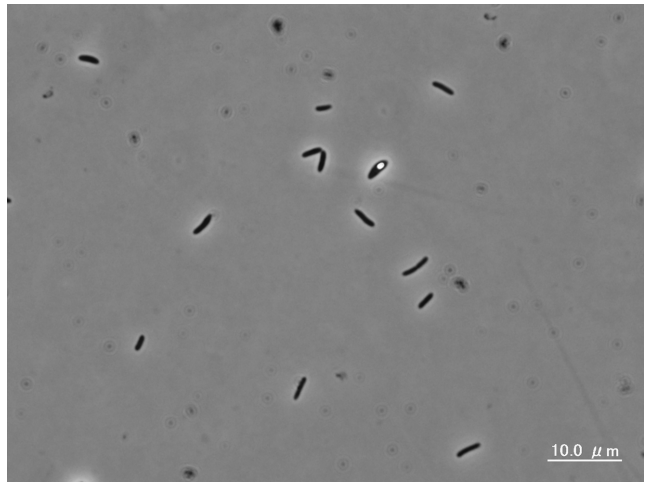

Fig. 1. Phase contrast photomicrograph of strain Tc2A^T grown on oxaloacetate.

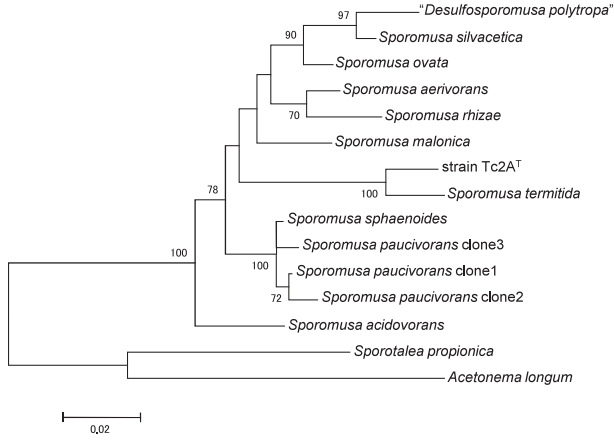

Fig. 2. Phylogenetic relationship based on 16S rRNA gene sequences of strain Tc2A^T and relative species belonging to the genus *Sporomusa*.

Unambiguously aligned 1,302 nucleotide sites, corresponding to positions 102−1388 in *Escherichia coli*, were used for the analysis. A maximum-likelihood tree was constructed with a general time reversible model assuming gamma distribution and invariable sites. 100 bootstrap resamplings were performed. Only bootstrap values $\geq$ 70% are shown.

Table 1. Differential characteristics of strain Tc2A^T and validly described *Sporomusa* species.

Characteristics	1	2	3	4	5	6	7	8	9	10
Cell width (μm)	0.5−0.7	0.5−0.8	0.6−0.7	0.7−1.0	0.5−0.8	0.7−1	0.4−0.7	0.7	0.7	0.8
Cell length (μm)	1.9−3.1	2−8	1.3−7	1−5	2−4	2−8	2−3	2.6−4.8	3.5	3.5
Endospores	+	+	+	+	+	+	−	+	+	+
Catalase	+	+	+	w	+	ND	−	−	−	−
Utilization of:										
H_2/CO_2	+	+	+	+	+	+	+	+	+	+
Fructose	+	−	−	+	−	+	−	+	+	−
Glycerol	+	−	−	−	+	+	+	+	+	ND
Mannitol	+	+	+	−	−	ND	−	ND	−	ND
Citrate	−	+	+	−	−	−	−	+	−	+
Fumarate	+	−	+	−	−	+	−	+	+	ND
Malate	+	−	+	ND	ND	+	−	+	ND	ND
Malonate	−	w	ND	ND	ND	−	ND	+	ND	ND
Methanol	+	+	+	+	+	+	+	+	+	ND
Propanol	+	−	ND	+	+	−	+	+	ND	ND
Sarcosine	−	+	ND	+	+	ND	−	ND	ND	ND
DNA G+C content (mol%)	51.0	48.6	ND	41.3−42.2	46.7−47.4	42	47.1	44.1±2	42.7±0.4	ND

Abbreviations: 1, strain Tc2A^T; 2, *S. termitida* strain JSN-2^T (Breznak et al., 1988); 3. *S.aerivorans* strain TmAO3^T (Boga et al., 2003); 4. *S. ovata* strain H1^T (Möller et al., 1984); 5, *S. sphaeroides* strain E^T (Möller et al., 1984); 6, *S. acidovorans* strain Mol^T (Ollivier et al., 1985); 7, *S. paucivorans* strain X^T (Hermann et al., 1987); 8, *S. malonica* strain WoGl2^T (Dehning et al., 1989); 9, *S. silvacetica* strain DG-1^T (Kuhner et al., 1997); 10, *S. rhizae* strain RS^T (Gößner et al., 2006). +, positive; −, negative; w, weakly positive; ND, not determined.

as a growth substrate. On the other hand, strain Tc2A^T differed from *S. termitida* and *S. aerivorans* with regard to its ability to grow on fructose and glycerol.

Based on the phenotypic, chemotaxonomic, and phylogenetic distinctiveness, we propose that the strain Tc2A^T should be classified as a type strain of the novel species, *Sporomusa intestinalis* sp. nov. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Tc2A^T is AB206386.

Description of *Sporomusa intestinalis* sp. nov.

Sporomusa intestinalis (L. n. intestinum, gut, intestine; L. suff. -alis. suffix denoting pertaining to; in.tes.ti.nal'is. N. L. fem. adj. *intestinalis* intestinal, pertaining to the intestine)

Cells are straight to slightly curved rods, 0.5−0.7 μm wide and 1.9−3.1 μm long. The cells occur singly, in pairs, and sometimes in chains. Gram- and KOH-reactions are negative. Anaerobic. Slowly motile. White to pale brown colonies, approximately 1 mm in diameter, were formed on the solidified medium with H_2/CO_2 after about 30 days' incubation. Endospores are formed. Catalase activity is positive. It grows at 15−45°C (optimum 35−37°C) and at pH 5.9−8.3 (optimum at pH 7.0). Growth occurs at NaCl concentration between 0−2% (optimum 0%) and at KCl concentration between 0−3% (optimum 0−0.5%). Cells utilize H_2/CO_2 (weakly), acetoin, betaine, 2,3-butanediol, *n*-butanol (weakly), ethyleneglycol, ethanol, ethanolamine, formate, fumarate, fructose, glutamate, glycerol, glycine (weakly), 3-hydroxybutyrate, lactate, malate, mannitol, methanol, oxaloacetate, 1,2-propanediol, *n*-propanol (weakly), pyruvate, succinate, syringate, vanillate, and 3,4,5-trimethoxybenzoate as carbon and energy sources. Acetate is the primary fermentation product. Cells does not utilize alanine, arabinose, aspartate, cellobiose, choline, citrate, 2-deoxyglucose, *N*,*N*-dimethylethanolamine, *N*,*N*-dimethylglycine, dulcitol, fucose, galactose, galacturonate, glucose, glucuronate, glyoxylate, lactose, malonate, maltose, melibiose, mannose, myo-inositol, rhamnose, raffinose, ribose, sarcosine, serine, sorbitol, sucrose, trehalose, trimethlamine, or xylose. Sulfate and nitrate are not utilized as an electron acceptor. Yeast extract is not essential but stimulates growth.

The DNA G+C content of the type strain is 51.0 mol%.

The type strain, Tc2A^T (JCM13218=DSM17189), was isolated from the gut content of a wood/soil interface-feeding higher termite, *Termes comis* (Termitinae) in Thailand.

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