Pectinatus sottacetonis sp. nov., isolated from a commercial pickle spoilage tank

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A strictly anaerobic, Gram-stain-negative, non-spore-forming, motile bacterium, designated strain FSRU B0405^T, was isolated from a commercial pickle spoilage tank and characterized by biochemical, physiological and molecular biological methods. Analyses of the 16S rRNA gene sequence of strain FSRU B0405^T showed affiliation to the class *Negativicutes* in the phylum *Firmicutes*, with the closest relatives being the type strains of *Pectinatus haikarae* (96 %) and *Pectinatus brassicae* (95 %). In maximum-likelihood and neighbour-joining phylogenetic trees, strain FSRU B0405^T clustered definitively (in 100 % of bootstrapped trees) within the genus *Pectinatus*, but not specifically with any characterized species within this genus. Strain FSRU B0405^T was a slightly curved rod, varying from 3 to 30 μm in length, motile with a distinctive X-wise movement, having flagella only on the concave side of the cell. The isolate produced acetate and propionate from fructose and glucose as major metabolites similar to type strains of species of the genus *Pectinatus*. The major fatty acids were $C_{11:0}$, $C_{13:0}$, $C_{15:0}$, $C_{13:0}$ 3-OH, $C_{17:1}$ and $C_{18:1}\omega 11t$. Strain FSRU B0405^T differed from the pickle wastewater strain, *Pectinatus brassicae* TY^T, due to its lack of susceptibility to vancomycin, acetoin production, growth temperature range, acid production from adonitol, erythritol, glycerol, inositol, lactose, maltose, mannose, ribose, salicin, sorbitol, trehalose and xylitol and lack of hydrolysis of milk. Strain FSRU B0405^T could be differentiated from other species of the genus *Pectinatus* both phenotypically and genetically. The results indicate that strain FSRU B0405^T represents a novel species of the genus *Pectinatus*, for which the name *Pectinatus sottacetonis* sp. nov. is proposed. The type strain is FSRU B0405^T (=ATCC BAA-2501^T=VTT E-113163^T). An emended description of the genus *Pectinatus* is also provided.

At the time of writing, the genus *Pectinatus* (Lee *et al.*, 1978; emend. Juvonen & Suihko, 2006) comprises five species with validly published names that are affiliated with the class *Negativicutes* in the phylum *Firmicutes* (Marchandin *et al.*, 2010). Until recently, species of the genus *Pectinatus* were only associated with spoiled beer and brewery environments (for a review, see Haikara & Juvonen, 2009). *Pectinatus cerevisiiphilus* was first described as a strictly anaerobic Gram-negative bacterium in spoiled beer (Lee *et al.*, 1978; emend. Schleifer *et al.*, 1990), followed by *Pectinatus frisingensis* (Schleifer *et al.*, 1990) and more recently *Pectinatus haikarae* (Juvonen & Suihko, 2006). Gonzalez *et al.* (2005) described a non-beer-associated species *Pectinatus portalensis* from a winery wastewater treatment plant. However, the cultures cited as the type strain of the species do not conform to the original description, and Vereecke and Arahal (2008) have requested that the Judicial Commission places the name *Pectinatus portalensis* on the list of rejected names if a suitable replacement type strain is not found or a neotype is not proposed within two years following the publication of their request. Another wastewater-related species *Pectinatus brassicae* was recently found in salty pickle wastewater, widening the known habitats of members of the genus *Pectinatus* (Zhang *et al.*, 2012).

Species of the genus *Pectinatus* are common spoilage bacteria in unpasteurized packaged beer, causing turbidity and off-flavours. These off-flavours are caused by the

Abbreviation: FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Pectinatus sottacetonis* FSRU B0405^T is JF280084.

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Two supplementary tables are available with the online version of this paper.

production of propionic and acetic acids and sulfur compounds such as hydrogen sulfide gas. Species of the genus *Pectinatus* have emerged as spoilage bacteria with new anaerobic beer filling techniques and with the shift towards 'natural' methods without pasteurization (Haikara & Helander, 2006). Beer spoilage by species of the genus *Pectinatus* is highly damaging due to noxious off-flavours produced that make the product unfit for consumption (Lee, 1994).

In August 2009, a Pectinatus-like strain, FSRU B0405^T, was isolated using reduced peptone yeast lactate (PYL) medium (Bacto peptone, 10 g l^{-1}; yeast extract, 10 g l^{-1}; sodium L-lactate, 20 g l^{-1}; vancomycin, 5 μg ml^{-1}) from a commercial pickle tank in eastern North Carolina, USA after spoilage had occurred. This was, to our knowledge, the second report of recovery of a species of the genus *Pectinatus* from commercial pickles and vegetable wastewater (Zhang *et al.*, 2012). The cucumber fermentation spoilage brine was at pH 4.9, with 4.3 % NaCl, 38 mM acetic acid, 46 mM propionic acid and 48 mM butyric acid (Franco *et al.*, 2012). Increased turbidity and rotten egg odour were noted in the sample. Lactic acid, succinic acid, malic acid, ethanol, glycerol, fructose and glucose were not detected. This 8000 gallon, outdoor vegetable brining tank, containing cucumber pieces such as nubs and relish, had not been air purged for over 12 weeks. Standard cucumber fermentations have low pH (3.3), 0.3 mg dissolved oxygen l^{-1} and a redox potential of approximately 350 mV and no propionic or butyric acids (Franco *et al.*, 2012). Spoiling cucumber fermentations may contain propionic acid and butyric acid, have pH values above 4.0 (Fleming *et al.*, 1989; Kim & Breidt, 2007) and dissolved oxygen (2.2 mg l^{-1}) values with a negative redox potential (−139 mV), indicative of reducing conditions (Franco *et al.*, 2012). It is believed that under reducing conditions, oxygen is scavenged by free radicals.

Strain FSRU B0405^T was identified by 16S rRNA gene cloning and sequencing of DNA from spoilage brine treated with propidium monoazide to detect only viable bacterial cells (Nocker & Camper, 2006; Pan & Breidt, 2007). Other micro-organisms detected in the spoilage community included *Clostridium sordellii*, *Acidaminococcus fermentans*, *Dialister micraerophilus*, members of the genera *Lactobacillus*, *Pediococcus*, and *Pseudomonas* and yeasts of the genus *Pichia* (F. Breidt, unpublished data).

Strain FSRU B0405^T and four other species of the genus *Pectinatus* [*Pectinatus brassicae* (DSM 24661^T); *Pectinatus cerevisiiphilus* (VTT E-79103^T); *Pectinatus frisingensis* (VTT E-79100^T) and *Pectinatus haikarae* (E-88329^T)] were anaerobically cultured at 30 °C in FCJ and peptone yeast fructose media (PYF). The FCJ was prepared by fermentation of *Lactobacillus plantarum* (MOP3) at 10^6 c.f.u. ml^{-1} on a 50 : 50 pack-out ratio of size 2B cucumbers with 0 % NaCl, 18 mM $Ca(OH)_2$ and 53 mM glacial acetic acid (Fleming *et al.*, 1995). Cucumbers and brine were homogenized in a Waring blender. Slurry was frozen and then thawed prior to use, centrifuged at 13 000 ***g*** for 20 min (Sorval), the supernatant was aspirated and filtered with a 0.45 μM vacuum filter unit (final pH 5.0). Peptone yeast fructose medium (Juvonen *et al.*, 1999) (5.0 g peptone, 5.0 g tryptone, 10.0 g yeast extract, 5.0 g fructose, 2.0 g Na_2HPO_4, 1.0 ml Tween 80 and 5.0 g cysteine HCl in1 l distilled water) was reduced in an anaerobic chamber (Coy Laboratory Products) for at least 24 h prior to inoculation with isolates of members of the genus *Pectinatus*.

When observed using phase-contrast and light microscopy, cells of strain FSRU B0405^T grown on anaerobic PYL-vancomycin medium were Gram-stain-negative and exhibited a distinctive X-shape during movement characteristic of members of the genus *Pectinatus*. FSRU B0405^T cells were imaged by scanning electron microscopy (SEM) and exhibited diverse morphologies as described previously (Haikara & Helander, 2006) (Figs. 1 and 2). Chains of rods and round cell forms were also present. Characteristic comb-like flagella were present but broken due to SEM preparation. Older, elongated cells had snake-like movement when observed under light microscopy.

Pickle spoilage brine aliquots of 10 ml were centrifuged (Sorvall, Thermo Fisher Scientific) at 2 750 ***g*** for 10 min at 4 °C, washed in saline solution and centrifuged as described above. Pellets were resuspended in 490 μl sterile saline solution and treated with propidium monoazide (PMA) to eliminate dead bacterial and extracellular DNA (Pan & Breidt, 2007). A 10 μl volume of 2.5 mM PMA stock solution (1.0 mg of PMA in 780 μl 20 % DMSO) (Biotium) was added to 490 μl spoilage pellet in saline (final concentration 50 μM PMA). Suspensions were vortexed briefly and placed in the dark for 5 min at room temperature. Then sample tubes were placed in ice slurry with tops open and exposed to a 650 W halogen lamp 20 cm above the tubes for 5 min. After this light exposure for cross-linking of the dyes with DNA, the cell suspensions were centrifuged at 3 300 ***g*** for 5 min at RT. The supernatant was removed and the pellet was treated with PMA again as described above. PMA-treated samples were stored as pellets at −20 °C until DNA extraction.

DNA was extracted using one of three different methods: DNeasy kit (Qiagen) Gram-negative protocol plus mutanolysin; DNeasy kit Gram-positive protocol plus mutanolysin and lysozyme or Power Soil DNA (MoBio) extraction kit which includes a bead beating step. The 16S rRNA sequence of strain FSRU B0405^T was determined using primers 8f (5′-AGAGTTTGA TCCTGGCTCAG-3′) and 1492r (5′-GGTT-ACCTTGTTACGACTT-3′) (Stackebrandt & Liesack, 1993) with the resulting amplicon sequenced in both directions using the same primers by commercial sequencing companies GeneWiz (South Plainfield, NJ, USA; http://www.genewiz.com) or Eton (San Diego, CA, USA; http://www.etonbio.com). Approximately 1517 base pairs were checked manually for errors and queried for similarities with BLAST analysis (Altschul *et al.*, 1990). The 16S rRNA gene sequence

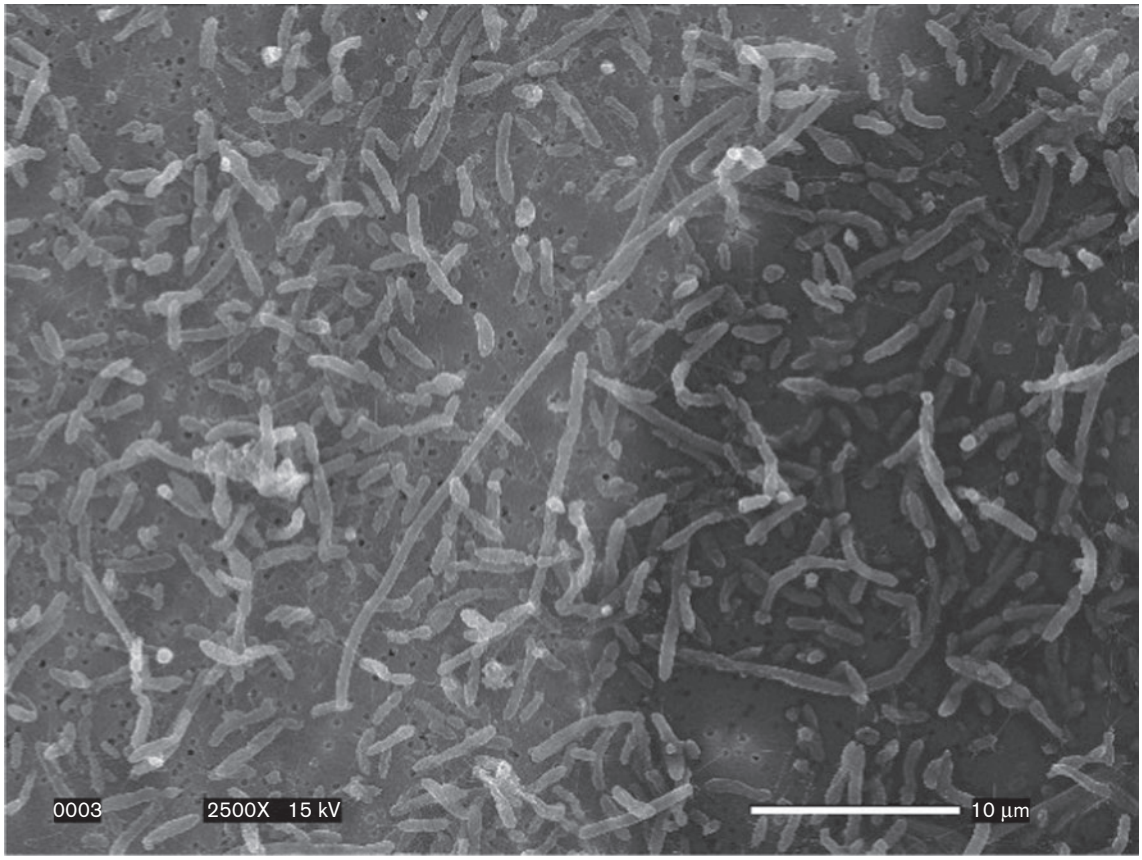

Fig. 1. Scanning electron micrograph of cells of *Pectinatus sottacetonis* sp. nov. showing different rod lengths and round cell forms. Bar, 10 µm.

of strain FSRU B0405^T (GenBank accession number JF280084) had 96 % sequence similarity (1439/1511 bp) with *Pectinatus haikarae* VTT E-88329^T (GenBank accession number DQ223731). The next highest match was with *Pectinatus brassicae* TY^T (GenBank accession number HM212531) at 95 % sequence similarity.

Maximum-likelihood and neighbour-joining phylogenetic trees were reconstructed using the Ribosomal Database Project website (http://rdp.cme.msu.edu/) and PHYLIP software (Fig. 3). Strain FSRU B0405^T clustered definitively (in 100 % of bootstrapped trees) within the genus *Pectinatus*, but not specifically with any characterized species within this genus.

Comparative biochemical and physiological characterizations using identical tests conditions were performed as previously described (Juvonen & Suihko, 2006). Antibiotic susceptibility tests were performed using triplicate tubes containing 3 ml PYL plus the concentrations of antibiotics listed. Turbidity was measured after 48–72 h of anaerobic growth at 30 °C (Table S1 available in IJSEM Online). Besides vancomycin, FSRU B0405^T was resistant to nisin (25 ng ml^{-1}), a lantibiotic used to suppress Gram-positive bacterial growth. This differs from previous findings where *Pectinatus cerevisiiphilus* was found to be highly sensitive to vancomycin (Helander *et al.*, 1994) and *Pectinatus frisingensis* was shown to be sensitive to nisin (Chihib *et al.*, 1999). FSRU B0405^T was sensitive to all other antibiotics tested (Table S1).

Growth was examined in de Man Rogosa Sharpe (MRS, Oxoid), peptone yeast extract fructose (PYF) and peptone yeast extract glucose (PYG) (Holdeman *et al.*, 1977) broth media and in selective medium for *Megasphaera* and *Pectinatus* (SMMP) (Lee 1994). FSRU B0405^T grew in filter-sterilized, light beer with 4 % v/v alcohol, approximately 10 bitterness units (BU), pH 4.2, using the method of Haakensen *et al.* (2007). For analysis of organic acids and sugars after growth on PYF or FCJ, samples (2 ml) were withdrawn aseptically at indicated times for HPLC and pH measurements. The pH was determined with an Accumet AR25 pH meter (Fisher). Organic acid and sugar concentrations were measured with a Thermo Separation Products HPLC (ThermoQuest) system. All species of the genus *Pectinatus* that grew on FCJ or PYF produced succinic, acetic and propionic acids from primary carbon sources, lactic acid or fructose, respectively (Table S2). No butyric acid was produced in either medium. Strain FSRU B0405^T produced acetate and propionate as major

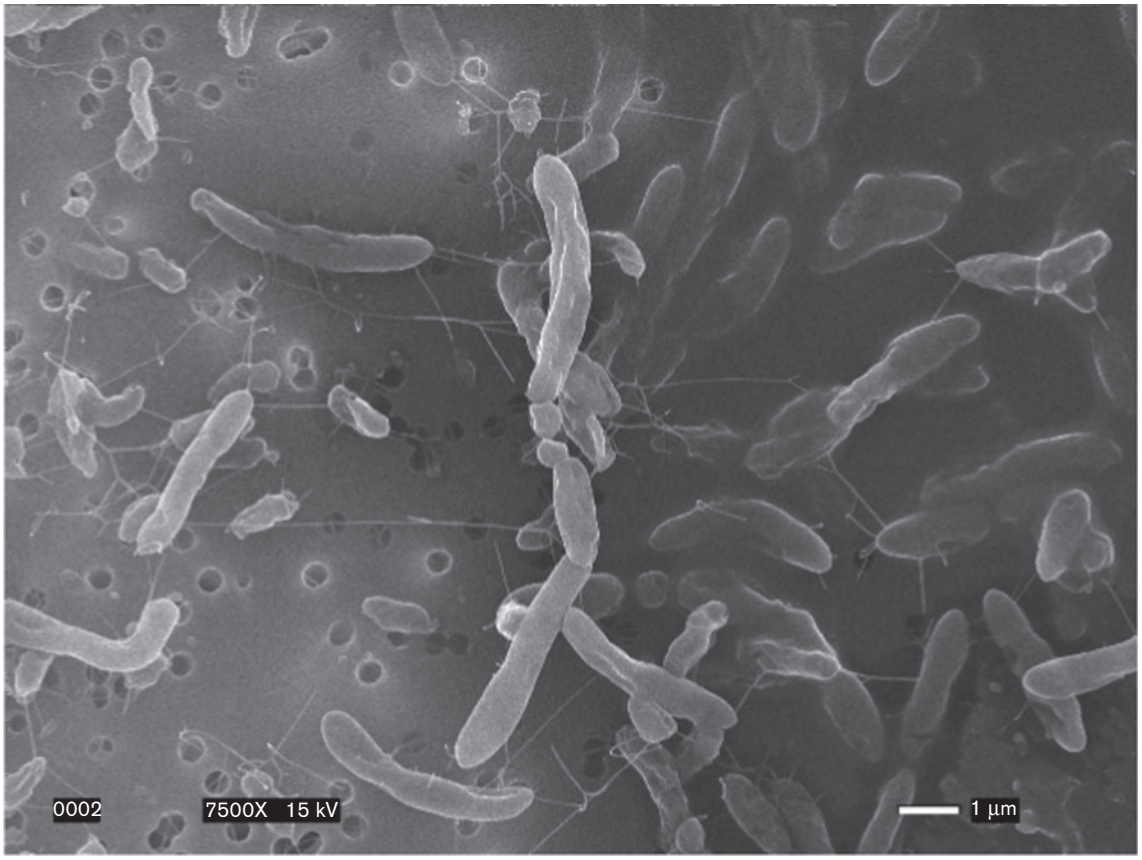

Fig. 2. Scanning electron micrograph of cells of *Pectinatus sottacetonis* sp. nov. showing shorter, curved rod forms with flagellae and round forms. Bar, 1 μm.

metabolites similarly to type strains of species of the genus *Pectinatus*. Moreover, H_2S and acetoin production was detected in PYF medium (Table 1). The observed metabolite profile supports affiliation of FSRU B0405^T to the genus *Pectinatus* (Schleifer *et al.*, 1990; Juvonen & Suihko, 2006). *Pectinatus brassicae* and *Pectinatus haikarae* were not able to grow on FCJ. After 7 days culture, the pH of FCJ rose from 4.9 to 5.4, perhaps due to the different pK_a values of the acids as lactic acid was converted to acetic and propionic acids. After 72 h growth, the pH of PYF media dropped from pH 4.3 to 4.1, presumably due to the production of acetic and propionic acids from fructose. The pH growth range for FSRU B0405^T was pH 5.0–8.0 (Table 1) cultured in PYL broth [Na-L-lactate (20 g l^{-1}), yeast extract (10 g l^{-1}), Bacto peptone (10 g l^{-1})] or fermented cucumber juice.

Strain FSRU B0405^T differed from the pickle wastewater strain, *Pectinatus brassicae* TY^T, due to its lack of susceptibility to vancomycin, acetoin production, growth temperature range, acid production from adonitol, cellobiose, erythritol, glycerol, inositol, lactose, maltose, mannose, ribose, salicin, sorbitol, trehalose and xylitol and lack of hydrolysis of milk (Table 1). FSRU B0405^T was originally isolated from commercial pickle spoilage brine with 4.3 % NaCl.

For whole-cell fatty acids analysis, the bacterial strains were grown on PYF agar medium (Juvonen *et al.*, 1999) under anaerobic conditions for 72 h at 30 °C. Cells were collected under anaerobic conditions, tubes were closed tightly and frozen until used. Whole-cell fatty acid methyl esters (FAMEs) were prepared from 40–60 mg of wet cell material and analysed by GC according to the Sherlock Microbial Identification (MIDI) protocol. Peaks were automatically integrated, and the FAMEs were identified and quantified using the MIDI anaerobic BHBIL library version 3.8 (MIDI). The fatty acid profile of strain FSRU B0405^T was very similar to the profiles already described for other species of the genus *Pectinatus* (Table 2) (Haikara & Helander 1995; Zhang *et al.*, 2012). The typical major fatty acids, i.e. $C_{11:0}$, $C_{13:0}$, $C_{15:0}$, $C_{13:0}$ 3-OH (most probably misidentified as $C_{14:0}$ in MIDI), $C_{17:1}$ and $C_{18:1}\omega11t$ could also be detected in strain FSRU B0405^T. However, some minor differences could be observed (Table 2). Strain FSRU B0405^T did not contain $C_{18:0}$ but did contain $C_{14:1}\omega7c$DMA and $C_{16:1}\omega11c$ when compared with *Pectinatus cerevisiiphilus* VTT E-79103^T, *Pectinatus*

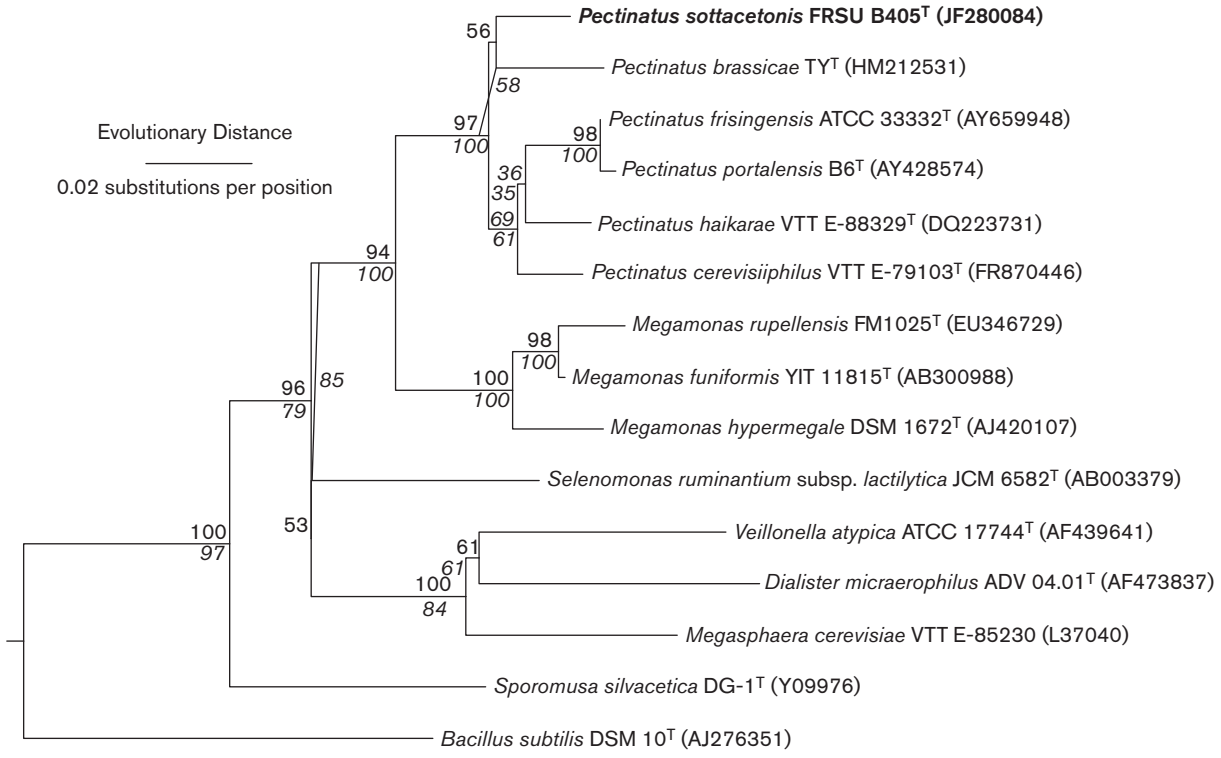

Fig. 3. Weighted neighbour-joining phylogenetic tree of 16S rRNA sequences (1517 bp) showing the position of *Pectinatus sottacetonis* sp. nov. within the Pectinatus–Sporomusa group of the family *Veillonellaceae*. The root of this tree was established using *Escherichia coli* (The type strain of *E. coli* used was ATCC 11775, the sequence is genbank accession number X80725) as the outgroup. Numbers are bootstrap values (rounded to the nearest percentage point) of 1000 weighted neighbour-joining trees (values above the branches) and maximum-likelihood trees (below the branches and in italics). Diagonal lines represent differences in consensus maximum-likelihood trees relative to the weighted neighbour-joining tree generated during bootstrap analysis. The *Pectinatus frisingensis* sequence is a partial sequence of only 472 nt.

frisingensis VTT E-79100^T and *Pectinatus haikarae* VTT E-88329^T.

The results of morphological characterization, 16S rRNA gene sequence comparison and metabolite analyses as well as fatty acid analysis demonstrate that strain FSRU B0405^T belongs to the genus *Pectinatus*. Moreover, the observed genetic and physiological/biochemical differences compared with the previously described species of the genus *Pectinatus* justify its description as a novel species. The proposed species name is *Pectinatus sottacetonis* sp. nov. with the type strain FSRU B0405^T (=ATCC BAA-2501^T=VTT E-113163^T) As the most recent emended description of the genus *Pectinatus* by Juvonen & Suihko (2006) does not take into consideration the properties of *Pectinatus brassicae* and strain FSRU B0405^T, an emended genus description is also proposed.

Emended description of the genus *Pectinatus* Lee *et al.* 1978

Pectinatus (Pec.ti.na′tus. L. part. adj. *Pectinatus* combed).

Cells are non-spore-forming slightly curved to helical rods, 0.4–1.0 × 2–50 μm or more, with rounded ends and a Gram-negative cell wall. They occur singly, in pairs or, rarely, in short chains. Cells are usually motile by means of comb-like flagellation which emanates from only one side of a cell. Organisms are strictly anaerobic mesophiles with a fermentative type of metabolism. Glucose and fructose are mainly metabolized to acetic and propionic acids. H_2S and occasionally minor amounts of succinic and lactic acid are also produced. Cells do not synthesize cytochrome oxidase or liquefy gelatin nor produce indole. Nitrate is not reduced. The DNA G+C content of members of this genus is 36–41 mol%. Members of this genus have been isolated from spoiled beer, brewery environments, spoiled pickles and wastewater.

The type species of the genus is *Pectinatus cerevisiiphilus* (Lee *et al.*, 1978; emend. Schleifer *et al.*, 1990).

Description of *Pectinatus sottacetonis* sp. nov.

Pectinatus sottacetonis [sot.ta.ce′to.nis. N.L. n. *sottaceto* (from Italian noun sottaceto), pickle; N.L. gen. n. *sottacetonis* of sottaceto, referring to the isolation of the type strain from a cucumber fermentation (pickle) spoilage tank].

Table 1. Differential characteristics of strain FSRU B0405^T and type strains of species of the genus *Pectinatus*

Strains; 1, FSRU B0405^T; 2, *Pectinatus brassicae* TY^T; 3, *Pectinatus haikarae* VTT E-88329^T; 4, *Pectinatus cerevisiiphilus* VTT E-79103^T; 5, *Pectinatus frisingensis* VTT E-79100^T. All data except that for *Pectinatus brassicae* (Zhang *et al.*, 2012) are from this study. All strains were positive for acid production from D-fructose, D-galactose and D-glucose. All strains were negative for acid production from glycogen, inulin, melezitose, raffinose and soluble starch and for production of oxidase and indole and hydrolysis of gelatin. W, Weakly positive; −, negative; +, positive; ND, not determined.

Characteristic	1	2	3	4	5
Catalase activity	−	−	−*[a]	+	−
Urease activity	−	−	−	+[b]	−[c]
Acetoin production	+	−	+[a]	+[c]	+[c]
Milk hydrolysis	−	+	+[a]	+	−[a]
L-Arginine hydrolysis	+	ND	−[a]	−[a]	−[a]
Susceptibility to vancomycin (5 μg ml^{-1})	−	+	−[a]	+[a]	−[a]
Growth NaCl (%) range	0–7	0–3	0–1	0–1	0–1
Growth pH range	5.0–8.0	3.5–8.5	4.0–8.0	4.0–8.0	3.5–8.0
Growth temperature range (°C)	15–37	10–40	15–30[a]	15–45	15–37
Acid production from:					
Adonitol (=ribitol)	+	−	+[a]	+[b]	+[d]
L-Arabinose	−	W	+[a]	+[b]	+[c,d]
Cellobiose	+	−	−[a]	+[b,c]	+[c,d]
Dulcitol	−	−	+[a]	+[b,d]	+[c,d]
DL-Erythritol	+	−	+[a]	+[b,c,d]	+[c, d]
Aesculin	−	W	−[a]	+[c]	−[c]
Glycerol	+	−	+[a]	+[b,c,d]	+[c,d]
Inositol	+	−	+[a]	−[b,c,d]	−[c,d]
Lactose	+	−	+[a]	+	−[a]
D-Maltose	+	−	−[a]	+	+[a]
D-Mannitol	+	+	+[a]	−[c,d]	+[c]
D-Mannose	+	−	+[a]	+[b]	+[c]
D-Melibiose	−	−	+[a]	+[c,d]	−[c,d]
N-acetylglucosamine	−	−	−[a]	W	+[c,d]
Rhamnose	−	−	+[a]	+[b,c,d]	+[c]
D-Ribose	+	−	+[a]	+[b,c,d]	+[c]
D-Salicin	+	−	−[a]	+	+
D-Sorbitol	+	−	+	+	+[c]
Sucrose	+	+	−	−[c]	+
Trehalose	+	−	−	−[c]	+
Xylitol	+	−	+[a]	+[c]	+[c]
D-Xylose	−	−	+[a]	+[c]	−[c]
Utilization of:					
Gluconate	+	ND	+[a]	+	−
Pyruvate	+	ND	−[a]	−	+
Succinate	+	ND	−[a]	−	−

*Results were congruent with those of: *a*, Juvonen and Suihko (2006); *b*, Lee *et al.* (1978); *c*, Schleifer *et al.* (1990); *d*, Haikara *et al.* (1981).

Cells are Gram-stain-negative to Gram-stain-variable, non-spore-forming, mesophilic, slightly curved rods. They occur singly and occasionally in pairs, forming long helical 'snakes' in stationary-phase. Rods are approximately 0.5 μm in width and range from 3 to 30 μm in length, depending on the age of the cultures. Cells are highly motile and exhibit a distinctive 'X-wise', flip-flop motion due to flagella being clustered on one side of the cell. Strictly anaerobic, they can only grow on degassed broth or on degassed agar plates after being spread and overlaid with additional solid media. Frozen cultures do not survive in 20 % glycerol (by volume) stocks stored at −80 °C, but require 7 % DMSO in liquid nitrogen for prolonged viability. Cultures grow well on PYL, PYF and MRS media between pH 5.0 and 8.0. Cultures are mesophilic, growing at 15–37 °C, but not at 10 °C or 45 °C, with an optimum at around 30 °C. Excellent growth (>5 on a McFarland scale) is obtained in PYF and PYG broth media after 2 days and moderate growth (2–3 on a McFarland scale) in MRS and SMMP media after 3 days at 30 °C. Cells grow in up to 7 % NaCl. Colonies on PYF and PYG plates after 3 days at 30 °C are flat to convex, light-yellowish, opaque and

Table 2. Whole-cell fatty acid contents (%) of strain FSRU B0405^T and the type strains of selected species of the genus *Pectinatus*

Strains; 1, FSRU B0405^T; 2, *Pectinatus haikarae* VTT E-88329^T; 3, *Pectinatus cerevisiiphilus* VTT E-79103^T; 4, *Pectinatus frisingensis* VTT E-79100^T.

Fatty acid*	1	2	3	4
Saturated				
$C_{9:0}$	0.9	0.04	0.1	0.6
$C_{10:0}$	0.5	0.2	0.3	0.5
iso-$C_{12:0}$			0.2	
$C_{15:0}$ 3-OH			0.2	0.5
$C_{11:0}$	12.3	9.4	11.7	12.2
$C_{12:0}$		0.6		1.0
$C_{13:0}$	7.4	13.7	5.4	5.7
$C_{14:0}$	0.4	0.5	0.6	1.0
$C_{14:0}$ DMA†	21.7	19.0	18.9	21.2
$C_{15:0}$	16.0	15.0	19.1	11.5
$C_{16:0}$	0.6	1.4	2.6	1.7
$C_{17:0}$	1.5	2.7	2.2	2.3
$C_{17:0}$ DMA	2.8	4.3	3.2	2.1
$C_{18:0}$		0.2	0.5	0.7
$C_{19:0}$	0.5	0.6	0.4	0.5
$C_{22:0}$ NHC	0.2	0.2	0.2	0.3
Unsaturated				
$C_{14:1}\omega7c$ DMA	0.7			
$C_{15:1}\omega9c/\omega8t$	0.2	0.3	0.3	0.3
$C_{16:1}\omega9c$		0.3	0.1	0.1
$C_{16:1}\omega11c$	0.3			
$C_{18:1}$ at 17.254 DMA‡	5.3	4.8	11.7	10.6
$C_{18:1}\omega9c$	3.2	5.3	3.7	1.9
$C_{18:1}\omega11t$	11.4	10.4	6.9	7.4
Summed features§				
2			0.2	0.6
4	4.5	4.1	3.1	3.5
5	1.3	1.1	1.3	1.4
6		0.2	0.4	1.0
8	4.1	3.2	4.9	9.4
10			0.5	0.5
Unknown fatty acid				
UN (ECL13.493)	1.9	1.0	1.3	1.3
UN (ECL17.223)	1.7	1.2		

*Names of the fatty acids as given by MIDI system.
†The fatty acid identified by MIDI as $C_{14:0}$ DMA is most probably $C_{13:0}$ 3-OH (Haikara and Helander, 2006).
‡Most probably $C_{17:1}$.
§Summed features are groups of two or three fatty acids that are not separable in the MIDI system: summed feature 2, $C_{12:0}$ 3-OH/$C_{13:0}$; summed feature 4, Un14.762/$C_{15:2}$/$C_{15:1}\omega7c$; summed feature 5, $C_{15:0}$/$C_{14:0}$ 3-OH; summed feature 6, anteiso-$C_{15:0}$ 3-OH/$C_{16:1}\omega7c$ DMA; summed feature 8, $C_{17:1}\omega9c$/$C_{17:2}$; summed feature 10, $C_{18:1}\omega11c/\omega9t/\omega6t$/Un17.834.

circular with lobate, erose margins and a diameter of 1–2 mm. Cells are resistant to vancomycin (5 μg ml^{-1}) and nisin (0.025 μg ml^{-1}) but susceptible to ampicillin, chloramphenicol, tetracycline, carbenicillin, erythromycin, streptomycin, kanamycin, nalidixic acid and rifampicin. Major products of glucose and fructose fermentation are propionic and acetic acid. Acetoin and H_2S are also produced. Cells are catalase-, oxidase- and urease-negative. Cells are negative for the production of indole and the hydrolysis of gelatin and milk. Cells are positive for acid production from D-fructose, D-galactose, D-glucose, adonitol, cellobiose, DL-erythritol, glycerol, inositol, lactose, maltose, D-mannitol, D-mannose, D-ribose, D-salicin, D-sorbitol, sucrose, trehalose and xylitol. Cells are positive for the utilization of gluconate, pyruvate and succinate and for L-arginine hydrolysis. Does not contain $C_{18:0}$ but contains $C_{14:1}\omega7c$ DMA and $C_{16:1}\omega11c$.

The type strain is FSRU B0405^T (=ATCC BAA-2501^T =VTT E-113163 ^{T}) isolated from a commercial pickle spoilage tank in North Carolina, USA.

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References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.

Chihib, N., Monnerat, L., Membre, J. M. & Tholozan, J. L. (1999). Nisin, temperature and pH effects on growth and viability of *Pectinatus frisingensis*, a Gram-negative, strictly anaerobic beer-spoilage bacterium. *J Appl Microbiol* **87**, 438–446.

Fleming, H. P., Daeschel, M. A., McFeeters, R. F. & Pierson, M. D. (1989). Butyric acid spoilage of fermented cucumbers. *J Food Sci* **54**, 636–639.

Fleming, H. P., McDonald, L. C., McFeeters, R. F., Thompson, R. L. & Humphries, E. G. (1995). Fermentation of cucumbers without sodium chloride. *J Food Sci* **60**, 312–315.

Franco, W., Pérez-Díaz, I. M., Johanningsmeier, S. D. & McFeeters, R. F. (2012). Characteristics of spoilage-associated secondary cucumber fermentation. *Appl Environ Microbiol* **78**, 1273–1284.

Gonzalez, J. M., Jurado, V., Laiz, L., Zimmermann, J., Hermosin, B. & Sainz-Jimenez, C. (2004). *Pectinatus portalensis* sp. nov. In *Validation of publication of new names and new combinations previously effectively published outside the IJSEM*, List no. 102. *Int J Syst Evol Microbiol* **55**, 547–549.

Haakensen, M. C., Butt, L., Chaban, B., Deneer, H. & Ziola, B. (2007). *horA*-Specific real-time PCR for detection of beer-spoilage lactic acid bacteria. *J Am Soc Brew Chem* **65**, 157–165.

Haikara, A., Penttila, L., Enari, T.-M & Lounatmaa, K. (1981). Microbiological, biochemical, and electron microscopic characterization of a Pectinatus strain. *Appl. Environ. Microbiol.* **41**, 511–517.

Haikara, A. & Helander, I. M. (1995). Cellular fatty acyl and alkenyl residues in *Megasphaera* and *Pectinatus* species: contrasting profiles and detection of beer spoilage. *Microbiology* **141**, 1131–1137.

Haikara, A. & Helander, I. (2006). *Pectinatus*, *Megashaera* and *Zymophilus*. In *The Prokaryotes*, 3rd edn.vol.4 pp. 965–981. Edited by

M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt. New York: Springer.

Haikara, A. & Juvonen, R. (2009). Genus XV. *Pectinatus*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn. pp. 1094–1099. Edited by P. De Vos, G. Garrity, D. Jones, N. R. Krieg, W. Ludwig, F. A. Rainey, K.-H. Schleifer & W. B. Whitman. New York: Springer.

Helander, I. M., Kilpeläinen, I., Vaara, M., Moran, A. P., Lindner, B. & Seydel, U. (1994). Chemical structure of the lipid A component of lipopolysaccharides of the genus *Pectinatus*. *Eur J Biochem* **224**, 63–70.

Holdeman, L. V., Cato, E. P. & Moore, W. E. C. (1977). *Anaerobe Laboratory Manual*, 4th edn. Blacksburg, VA: Virginia Polytechnic Institute and State University.

Juvonen, R. & Suihko, M.-L. (2006). *Megasphaera paucivorans* sp. nov., *Megasphaera sueciensis* sp. nov. and *Pectinatus haikarae* sp. nov., isolated from brewery samples, and emended description of the genus *Pectinatus*. *Int J Syst Evol Microbiol* **56**, 695–702.

Juvonen, R., Satokari, R., Mallison, K. & Haikara, A. (1999). Detection of spoilage bacteria in beer by PCR. *J. Am. Soc. Brew. Chem* **57**, 99–103.

Kim, J. & Breidt, F. (2007). Development of preservation prediction chart for long term storage of fermented cucumber. *Korean J Life Sci* **17**, 1616–1621.

Lee, S. Y. (1994). SMMP: A medium for selective isolation of *Megasphaera* and *Pectinatus* from the brewery. *J Am Soc Brew Chem* **52**, 115–119.

Lee, S. Y., Mabee, M. S. & Jangaard, N. O. (1978). *Pectinatus*, a new genus of the family *Bacteroidaceae*. *Int J Syst Bacteriol* **28**, 582–594.

Marchandin, H., Teyssier, C., Campos, J., Jean-Pierre, H., Roger, F., Gay, B., Carlier, J.-P. & Jumas-Bilak, E. (2010). *Negativicoccus succinicivorans* gen. nov., sp. nov., isolated from human clinical samples, emended description of the family *Veillonellaceae* and description of *Negativicutes* classis nov., *Selenomonadales* ord. nov. and *Acidaminococcaceae* fam. nov. in the bacterial phylum *Firmicutes*. *Int J Syst Evol Microbiol* **60**, 1271–1279.

Nocker, A. & Camper, A. K. (2006). Selective removal of DNA from dead cells of mixed bacterial communities by use of ethidium monoazide. *Appl Environ Microbiol* **72**, 1997–2004.

Pan, Y. & Breidt, F., Jr (2007). Enumeration of viable *Listeria monocytogenes* cells by real-time PCR with propidium monoazide and ethidium monoazide in the presence of dead cells. *Appl Environ Microbiol* **73**, 8028–8031.

Schleifer, K. H., Leuteritz, M., Weiss, N., Ludwig, W., Kirchhof, G. & Seidel-Rüfer, H. (1990). Taxonomic study of anaerobic, Gram-negative, rod-shaped bacteria from breweries: emended description of *Pectinatus cerevisiiphilus* and description of *Pectinatus frisingensis* sp. nov., *Selenomonas lacticifex* sp. nov., *Zymophilus raffinosivorans* gen. nov., sp. nov., and *Zymophilus paucivorans* sp. nov. *Int J Syst Bacteriol* **40**, 19–27.

Stackebrandt, E. & Liesack, W. (1993). Nucleic acids and classification. In *Handbook of new bacterial systematics*, pp. 152–189. Edited by M. Goodfellow & A. G. O'Donnell London: Academic Press.

Vereecke, C. & Arahal, D. R. (2008). The status of the species *Pectinatus portalensis* Gonzalez *et al.* 2005. Request for an Opinion. *Int J Syst Evol Microbiol* **58**, 1507.

Zhang, W. W., Fang, M. X., Tan, H. Q., Zhang, X. Q., Wu, M. & Zhu, X. F. (2012). *Pectinatus brassicae* sp. nov., a Gram-negative, anaerobic bacterium isolated from salty wastewater. *Int J Syst Evol Microbiol* **62**, 2145–2149.