Saccharicrinis carchari sp. nov., isolated from a shark, and emended descriptions of the genus Saccharicrinis and Saccharicrinis fermentans

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A Gram-stain-negative, facultatively anaerobic, gliding, yellow-pigmented bacterium, designated SS12T, was isolated from shark gill homogenate and characterized using a polyphasic approach. The strain was catalase-positive and oxidase-negative. Optimal growth occurred at 28–30 °C, pH 7.0–7.5 and in the presence of 2–4 % (w/v) NaCl. The DNA G+C content was 40.0 mol%.

The strain contained MK-7 as the prevailing menaquinone; iso-C15 : 0 and anteiso-C15 : 0 as the major cellular fatty acids; and phosphatidylethanolamine and an unknown lipid as the predominant polar lipids. Comparative analysis of 16S rRNA gene sequences demonstrated that the novel isolate showed the highest sequence similarity (94.68 %) to Saccharicrinis fermentans DSM 9555T and the sequence similarities among the type strains of all other species studied were less than 92 %. A phylogenetic tree, based on 16S rRNA gene sequences, showed that strain SS12T and Saccharicrinis fermentans DSM 9555T formed a distinct cluster within the family Marinilabiliaceae. On the basis of its phylogenetic position and phenotypic traits, strain SS12T represents a novel species of genus Saccharicrinis, for which the name Saccharicrinis carchari sp. nov. is proposed. The type strain is SS12T (=CICC 10590T=DSM 27040T). Emended descriptions of the genus Saccharicrinis and Saccharicrinis fermentans are also provided.

In recent years, many new taxa have been described in the class Bacteroidia, a new family Marinilabiliaceae in the class was also proposed by Ludwig et al. (2011). At the time of writing, the family Marinilabiliaceae contains 10 genera namely Alkaliflexus, Alkalitalea, Anaerophaga, Geofilum, Mangroviolae, Marinilabia, Natronoflexus, Thermophilus, Carboxylibirga and Saccharicrinis, with Marinilabia as the type genus. For a long time, the genera Prolixibacter, Marinifilum and Sunxiuinia (Holmes et al., 2007; Na et al., 2009; Ruivira et al., 2013; Qu et al., 2011; Takai et al., 2013) appeared to represent deep lineages in the class Bacteroidia but their affiliation with recognized families were ambiguous. Recently, another novel family, Prolixibacteraceae, was proposed to accommodate the genera Prolixibacter, Sunxiuinia and a newly described genus Mangrovibacterium (Huang et al., 2014).

The taxonomy of the genus Cytophaga has a complicated history, with several species transferred to and from this genus. It has long been thought that a new genus should be created to accommodate [Cytophaga] fermentans (Suzuki et al., 1999). Ludwig et al. (2011) suggested that [Cytophaga] fermentans should be reclassified as a novel genus that is associated with the family Marinilabiliaceae. However, due to its unique clading and the fact that 16S rRNA gene sequence evidence was only available from a single strain, it could not be ascertained until Yang et al. (2014) proposed to reclassify [Cytophaga] fermentans as Saccharicrinis fermentans gen. nov., comb. nov. within the family Marinilabiliaceae.

The aim of this study was to elucidate the taxonomic status of a novel strain, SS12T. Phylogenetic analysis of the 16S rRNA gene sequence revealed a clear affiliation of strain SS12T with members of the family Marinilabiliaceae, with Saccharicrinis fermentans DSM 9555T as the closest phylogenetic relative. On the basis of phylogenetic, physiological and biochemical parameters, we are of the opinion that strain SS12T represents a novel species of genus Saccharicrinis.

The novel strain was isolated from gill homogenate of a dead shark (Cetorhinus maximus) that was caught from the Yellow Sea, China by fishermen. The homogenate was collected from the gills of a dead shark (Cetorhinus maximus) that was caught from the Yellow Sea, China by fishermen. The homogenate was collected from the gills of a dead shark (Cetorhinus maximus) that was caught from the Yellow Sea, China by fishermen. The homogenate was collected from the gills of a dead shark (Cetorhinus maximus) that was caught from the Yellow Sea, China by fishermen.
diluted serially in sterile distilled water and samples of each serial dilution were spread on marine agar 2216 (MA; Difco) and incubated at 28 °C for 7 days. A yellow-pigmented bacterium, designated SS12T, was isolated and purified by sub-culturing on the same medium. Subsequently, strain SS12T was routinely grown on MA or marine broth 2216 (MB; Difco) at 28 °C and pure cultures were maintained as glycerol/water suspensions (20 %, v/v) at −80 °C or as lyophilized vials. Saccharicrinis fermentans DSM 9555T was obtained from the Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Unless stated otherwise, the two organisms were grown on MA under identical conditions for comparative purposes.

Cell morphology was observed using light microscopy (E600; Nikon) and Gram staining was performed as described by Smibert & Krieg (1994). Gliding motility was examined according to the method described by Bowman (2000) by using oil-immersion phase-contrast microscopy (AX70; Olympus). Oxidase activity was tested by using the bioMérieux oxidase reagent kit according to the manufacturer's instructions. Catalase activity was detected by pouring a 3 % H2O2 solution on bacterial colonies. Reduction of nitrate and the oxidation-fertilization test were performed as described by Dong & Cai (2001). For the nitrate-reducing test, the medium was amended with filter-sterilized seawater for strain SS12T and Saccharicrinis fermentans DSM 9555T, since they showed poor growth with the sea-salt-free basal medium. Hydrolysis of agar, starch, CM-cellulose, alginate, asesculin and Tween 80 were examined according to methods described by Dong & Cai (2001) by using MA medium. Halotolerance was tested on appropriately modified MA (seawater replaced with distilled water) at NaCl concentrations of 0–15 % (w/v, at intervals of 1 %). Growth ranges and optima of temperature were indicated by visible colonies on MA at 4, 8, 10, 12, 15, 20, 25, 28, 30, 33, 35, 37, 40, 42 and 45 °C. The pH range for growth was determined by adding 20 mM MES (for pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5) or CAPSO (pH 9.0, 9.5 and 10.0) (Sangon) to MB medium as a buffer system. The substrate-oxidation profile was obtained by using Biolog GEN III microplates following the manufacturer's protocol. API 50 CHB fermentation kits (bioMérieux) were used for determining carbohydrate production from carbohydrates. Various biochemical tests and additional enzyme activities were determined using API 20E strips and the API ZYM system (bioMérieux) as recommended by the manufacturer, except that cells were suspended in 3 % (w/v) NaCl solution. Susceptibility of both strains to antimicrobial agents was tested by the disc diffusion method, as described by Jorgensen et al. (1999), using antibiotic-impregnated discs (Tianhe). A modification was that the tests were done on MA, since strain SS12T showed poor growth on Mueller–Hinton agar. Each set of experiments was repeated twice.

PCR amplification of 16S rRNA gene was carried out using two universal primers: 27f (5'-AGAGTTTGTATCMTGGCTCAG-3') and 1492r (5'-TACGTYTACCTTGGTTACGA-C-3') (Jordan et al., 2007), and the purified PCR product was ligated into the pGM-T vector (Tiangen) and cloned according to the manufacturer's instructions. Sequencing primers T7 (5'-TAATACGACTCCTATAGGG-3') and SP6 (5'-ATTTAGGTACACTATAG-3') were used for sequencing of the cloned PCR products. The determined 16S rRNA gene sequence was submitted to the GenBank database and preliminary screening for similarity was done with the BLAST algorithm and also confirmed by accessing the EzTaxon-e BLAST analysis (Kim et al., 2012). Multiple alignments of sequences were performed using CLUSTAL X (version 1.81) (Thompson et al., 1997) and the ambiguous and unalignable bases were manually omitted. Phylogenetic trees were inferred with the neighbour-joining and maximum-likelihood methods implemented in the software package MEGA version 6 (Tamura et al., 2013) and the maximum-parsimony algorithm available in PAUP* (Swofford, 2002). Distances were calculated according to Kimura's two-parameter model (Kimura, 1980). Tree topologies were calculated by bootstrap analyses based on 1000 resamplings.

The nearly complete 16S RNA gene sequence (1483 nt) of strain SS12T was obtained. The neighbour-joining tree reconstructed from the Kimura's two-parameter distance matrix computed from the 16S RNA gene sequence alignment is shown in Fig. 1. Phylogenetic analysis revealed that strain SS12T shared 94.68 % 16S rRNA gene sequence similarity with the closest relative Saccharicrinis fermentans DSM 9555T, and followed by members of the genus Carboxybacterium (91.4–91.6 %) which was proposed by Yang et al. (2014). In addition to this, strain SS12T showed less than 90 % sequence similarity to the other representative members within the family Marinilabiliaceae. As clearly shown in Fig. 1, strain SS12T formed a monophyletic clade with Saccharicrinis fermentans DSM 9555T within the family Marinilabiliaceae, with a bootstrap value of 100 %. The tree topology was very similar for each of the phylogenetic methods employed (data not shown). The unique phylogenetic position of the strain and low sequence similarity values with respect to other recognized bacterial species studied led us to conclude that strain SS12T deserved the status of a novel species affiliated to the genus Saccharicrinis.

Genomic DNA was extracted and purified according to the method of Marmur (1961), using a commercial genomic DNA extraction kit (Tiangen), and the G + C content of DNA was determined by HPLC as described by Mesbah et al. (1989). Fatty acid compositions were determined as described by Sasser (1990) by using the Microbial Identification System (Microbial ID). Cultures for fatty acid analysis were incubated on MA slants at 28 °C for 4 days. Menaquinones were isolated by using the methods of Minnikin et al. (1984) and then separated by HPLC (Kroppenstedt, 1982). Polar lipids analysis was carried out by the Identification Service of the DSMZ, Braunschweig, Germany.

The DNA G + C content of strain SS12T was 40.0 mol%, similar to that of Saccharicrinis fermentans DSM 9555T. The DNA G + C content of strain SS12T was 40.0 mol%, similar to that of Saccharicrinis fermentans DSM 9555T.
Chemotaxonomic markers were also consistent with separating the isolate SS12T as a novel species of genus *Saccharicrinis*. Strain SS12T had the same characters of possessing MK-7 as *Saccharicrinis fermentans* DSM 9555\textsuperscript{T}. The predominant cellular fatty acids of both strain SS12T and *Saccharicrinis fermentans* DSM 9555\textsuperscript{T} were iso-C\textsubscript{15:0} and anteiso-C\textsubscript{15:0}, although there were some differences in the proportions and kinds of less abundant fatty acids. The two isolates shared lipid profiles consisting of the major compounds phosphatidylethanolamine (PE) and an unknown lipid (L3), as well as moderate and minor amounts of two phospholipids (PL1–2) and another two unknown lipids (L1–2), in spite of some quantitative differences being observed and a glycolipid (GL) was present in the polar lipid profile of strain SS12T but not in that of *Saccharicrinis fermentans* DSM 9555\textsuperscript{T}. (Fig. S1, available in the online Supplementary Material).

Despite these common chemotaxonomic traits demonstrated between the two isolates, strain SS12T differed sufficiently from *Saccharicrinis fermentans* DSM 9555\textsuperscript{T} on the basis of hydrolysis of urea but not agar; positive results for trypsin, α-glucosidase, α-fucosidase and N-acetyl-β-glucosaminidase activities, nitrate reduction and indole production; acid production from melezitose and N-acetylg glucosamine, but not from d-mannitol; and the ability to grow at 12 °C and oxidize different sole carbon sources in Biolog GEN microplates. Strain SS12T could also be readily distinguished from *Carboxylicivirga mesophila* MEBiC 07026\textsuperscript{T} and *Carboxylicivirga taeanensis* MEBiC 08903\textsuperscript{T} by its ability to produce α-fucosidase but not leucine arylamidase, and members of the latter two species cannot grow at 12 °C and are not motile by gliding. Criteria which are useful in distinguishing strain SS12T from its phylogenetically related neighbours are shown in Table 1.

The phylogenetic data, in conjunction with other phenotypic and chemotaxonomic characteristics, strongly suggest that strain SS12T represents a novel species in the genus *Saccharicrinis*, for which we propose the name *Saccharicrinis carchari* sp. nov. Emended descriptions of the genus *Saccharicrinis* and *Saccharicrinis fermentans* are also provided based on data obtained in this study.

**Description of Saccharicrinis carchari** sp. nov.

*Saccharicrinis carchari* (car.cha’ri. L. gen. n. carchari of a shark).
Table 1. Comparison of major features of strain SS12T with its phylogenetically related neighbours

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>Colony colour</td>
<td>Yellow</td>
<td>Bright yellow</td>
<td>Yellow</td>
<td>Yellow</td>
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<tr>
<td>Cell size (μm)</td>
<td>0.5–0.7 × 7–14</td>
<td>0.3–0.7 × 8–50</td>
<td>0.4–0.7 × 8.2–11.8</td>
<td>0.3–0.6 × 6.1–18.7</td>
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<tr>
<td>Gliding motility</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Growth at 12 °C</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>Indole production</td>
<td>+</td>
<td>–</td>
<td>V</td>
<td>–</td>
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<tr>
<td>Hydrolysis of:</td>
<td></td>
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<tr>
<td>Urea</td>
<td>+</td>
<td>–</td>
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<td>Agar</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Enzymic activities</td>
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<tr>
<td>Esterase(C4)</td>
<td>w</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Leucine arylamidase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Trypsin</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>α-Glucosidase</td>
<td>+</td>
<td>–</td>
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<td>+</td>
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<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>α-Fucosidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
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</table>

The characteristics are well-matched to the genus description of Saccharicrinis. Cells are facultatively anaerobic, chemo-organotrophic and motile by gliding. Gram-stain-negative, non-endospore-forming, slender, straight or slightly curved rods, approximately 0.5–0.7 μm wide and 7–14 μm long. Requires NaCl for growth. Catalase-positive and oxidase-negative. Colonies on MA are yellow-pigmented, circular to irregular-shaped and about 1 mm in diameter after 4 days of growth at 28 °C. Growth occurs at 10–40 °C and in the presence of 1–5 % (w/v) NaCl, with the optimum growth at 28–30 °C and with 2–4 % (w/v) NaCl. The optimum pH is between 7.0 and 7.5; growth does not occur below pH 6.5 or above pH 8.5. Starch, urea, CM-cellulose and aesculin are hydrolysed, but agar, gelatin, alginate and Tween 80 are not. Positive for nitrate reduction, Simmons’ citrate utilisation and indole production, but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, trypophan deaminase, H2S production and Voges–Proskauer reaction. Positive oxidations were observed with acetocetate acid, glucuronanilde, D-glucose 6-phosphate, D-fructose 6-phosphate, melibiose, methyl β-D-glucoside, D-salicin, N-acetyl-D-glucosamine, maltose, trehalose, sucrose and turanose in Biolog GEN III microplates. Acid is produced from D-xyllose, D-galactose, D-glucose, D-fructose, D-mannose, methyl α-D-glucopyranoside, N-acetylglycosamine, salicin, amygdalin, aesculin, cellobiose, maltose, lactose, sucrose, trehalose, melezitose, starch, glycogen, gentiobiose and potassium 5-ketoglutarate, but not from erythritol, D-arabinose, L-arabinose, D-ribose, L-xyllose, D-adonitol, methyl β-D-xylpyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, arbutin, melibiose, inulin, raffinose, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, potassium gluconate or potassium 2-ketoglucurate in API 50CH strips. According to API ZYM test results, alkaline phosphatase, esterase lipase (C8), trypsin, acid phosphatase, β-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-fucosidase activities are present, but lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, α-chymotrypsin, naphthol-AS-BI-phosphohydroxylase, Z-glucosidase, β-glucuronidase and α-mannosidase activities are absent. The reaction of esterase (C4) was weakly positive. Resistant to the following antimicrobial agents: neomycin, polymyxin B, streptomycin and trimethoprim, but sensitive to novobiocin, kanamycin, erythromycin, midecamycin, clindamycin, lincomycin, tobramycin and trimethoprim/sulfamethoxazole. The predominant cellular fatty acids (≥10 %) are iso-C15:0 and anteiso-C15:0. The main respiratory quinone is MK-7. The major polar lipids are phosphatidylethanolamine and an unknown lipid. In addition, a glycolipid, two unknown phospholipids and another two unknown lipids are present in moderate to minor amounts.

The type strain, SS12T (=CICC 10590T=DSM 27040T), was isolated from shark gill homogenate. The DNA G+C content of the type strain is 40.0 mol%.

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Emended description of the genus Saccharicrinis Yang et al. 2014

The description of the genus Saccharicrinis is as given by Yang et al. (2014) with the following amendments. Suitable for neutral pH or slightly alkalophilic. Membrane polar lipids included phosphatidylethanolamine and at least one unidentified lipid as major constituents.

Emended description of Saccharicrinis fermentans Yang et al. 2014

The description is the same as provided by Yang et al. (2014), with the following additions from the present study. Hydrolyses CM-cellulose but not alginate or Tween 80. Positive for Simmons’ citrate utilization, but negative for Voges–Proskauer reaction. N-acetyl-β-glucosaminidase activity is absent. In Biolog GEN III microplates, oxidizes a few carbon sources such as L-malic acid, glucuronamide and L-alanine. Acid is produced from D-xyllose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, amygdalin, aesculin, salicin, cellobiose, maltose, lactose, sucrose, trehalose, starch, glycogen, gentiobiose and potassium 5-ketogluconate, but not from glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, L-xyllose, D-adonitol, methyl β-D-xlyopyranoside, L-sorbose, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, arbutin, melibiase, inulin, melezitose, raffinose, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate or potassium 2-ketogluconate in API 50CH strips. Resistant to tetracycline, tobramycin, ciprofloxacin, kanamycin, novobiocin, midecamycin, clindamycin and lincomycin.

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