***Haloanaerobium fermentans* sp. nov., a strictly anaerobic, fermentative halophile isolated from fermented puffer fish ovaries**

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A strain of strictly anaerobic and moderately halophilic bacteria isolated from salted puffer fish ovaries was studied phenotypically, genotypically and phylogenetically. On the basis of its physiological and morphological characteristics, the new isolate is considered to be a member of the genus *Haloanaerobium*. It is a motile, rod-shaped, non-spore-forming, Gram-negative, obligate anaerobe that grows in the presence of 25% (w/v) NaCl. The optimum salt concentration for growth is 10% (w/v). It grows well at 15 and 45 °C, but not at 10 or 50 °C. The optimum temperature for growth is 35 °C. It grows at pH 6.0–9.0 and the optimum pH for growth is 7.5. It ferments *N*-acetylglucosamine, cellobiose, fructose, galactose, D-glucose, lactose, maltose, D-mannose, raffinose, D-ribose, sucrose and D-xylose. It ferments D-glucose with the production of hydrogen, carbon dioxide, ethanol and organic acids such as acetate, formate and lactate. 16S rRNA gene sequence information confirmed the phylogenetic position of the new isolate, strain R-9^T, as a member of the genus *Haloanaerobium*. DNA–DNA hybridization data revealed that isolate R-9^T exhibited low levels of reassociation (less than 30%) with previously described *Haloanaerobium* species. Based on these results, the new isolate appears to represent a new *Haloanaerobium* species, for which the name *Haloanaerobium fermentans* sp. nov. is proposed. The type strain is R-9^T (= JCM 10494^T).

Keywords: fermented seafood, halophilic fermentative bacteria, *Haloanaerobium fermentans*

INTRODUCTION

Members of the genus *Haloanaerobium* are widely distributed in surface saline ecosystems such as hypersaline lakes and subsurface ecosystems such as oil-fields. Eight species of this genus have been described previously. They have been isolated from various hypersaline environments: the Great Salt Lake (Zeikus *et al.*, 1983; Tsai *et al.*, 1995), Sivash Lake (Zhilina *et al.*, 1992), Retba Lake (Cayol *et al.*, 1994, 1995), gas-bearing sandstones and brine waters (Rengpipat *et al.*, 1988), petroleum reservoir fluid (Bhupathiraju *et al.*, 1994, 1999) and oil-injection water (Ravot *et al.*, 1997).

In a previous study, we described halophilic bacteria that grew only anaerobically in the manufacturing

process of a Japanese seafood, puffer fish ovaries fermented with rice-bran or *fugunoko nukaduke* (Kobayashi *et al.*, 1995), but the detailed characteristics of these anaerobes were not reported. In this study, we undertook a phenotypic, genotypic and phylogenetic characterization of R-9^T, an isolate of the new halophilic anaerobe that is representative of one of the dominant species in these fermented ovaries. The precise identification and phylogenetic position of the new isolate was determined by phenotypic characterization, 16S rRNA gene sequencing and DNA–DNA hybridization. On the basis of our results, we conclude that our isolate should be placed in a new species of *Haloanaerobium*.

METHODS

Bacterial strains and media. A new isolate (R-9^T) was obtained previously from salted puffer fish ovaries in the manufacturing process of a Japanese traditional food

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The DDBJ accession number for the 16S rRNA gene sequence of strain R-9^T (= JCM 10494^T) is AB023308.

product, *fugunoko nukaduke* (Kobayashi *et al.*, 1995). To prepare *fugunoko nukaduke*, puffer fish ovaries are salted for at least 6 months and the ovaries are then fermented naturally with rice-bran, fish sauce and *koji* for several years. To isolate and purify the new isolate, we used GYP agar containing (l⁻¹ distilled water): 10 g glucose, 10 g yeast extract, 5 g Bactopeptone, 2 g meat extract (Kyokuto), 2 g CH₃COONa·3H₂O, 5 g CaCO₃, 5 ml salt solution (l⁻¹: 40 g MgSO₄·7H₂O, 2 g MnSO₄·4H₂O, 2 g FeSO₄·7H₂O, 2 g NaCl), 0.25 g Tween 80, 200 g NaCl and 12 g agar. A GasPak anaerobic system (BBL) was used for cultivation. The type strains of *Haloanaerobium praevalens* (DSM 2228^T), *Haloanaerobium acetoethylicum* (DSM 3532^T), *Haloanaerobium saccharolyticum* subsp. *saccharolyticum* (DSM 6643^T), *Haloanaerobium saccharolyticum* subsp. *senegalense* (DSM 7379^T), *Haloanaerobium alcaliphilum* (DSM 8275^T), *Haloanaerobium lacusrosei* (DSM 10165^T) and *Haloanaerobium congolense* (DSM 11287^T) were obtained from the DSMZ (Braunschweig, Germany). *Haloanaerobium salsuginis* ATCC 51327^T was obtained from the ATCC (Manassas, VA, USA). Isolate R-9^T was stabbed into anaerobic bacterial culture medium (ABCM; Eiken) consisting of (l⁻¹ distilled water): 3 g glucose, 5 g yeast extract, 10 g peptone, 3 g soypeptone, 10 g tryptone, 2 g plant extract, 5 g soluble starch, 3 g meat extract, 2 g NaCl, 2.5 g K₂HPO₄, 0.005 g haemin, 0.3 g L-cysteine·HCl and 0.3 g sodium thioglycolate, supplemented with 10% (w/v) NaCl and 0.5% CaCO₃. The culture was maintained at 5 °C and subcultured every 6 months. The inocula used were prepared from 48- to 72-h-old cultures in ABCM medium supplemented with 10% (w/v) NaCl incubated at 30 °C under anaerobic conditions.

Cultural, physiological and biochemical characteristics. Gram staining was performed as described previously (Dussault, 1955). Cell morphology and motility were observed by light microscope. Cells for electron microscopy were stained with 2% (w/v) phosphotungstic acid. Micrographs were taken with a model Hitachi H-7000 electron microscope with an acceleration voltage of 75 kV. To test for the formation of endospores, growth was observed after pasteurization at 70 °C for 10 min. Catalase activity was tested with 3% (w/v) H₂O₂. Hydrogen sulfide production was determined with sulfide indole motility (SIM) medium (Eiken). Nitrate reduction was tested by using Gifu anaerobic medium (GAM; Nissui) supplemented with 0.1% KNO₃ and 0.15% agar. To test for the ability to ferment various carbohydrates, a basic medium (Oren *et al.*, 1984) containing 40 mM PIPES buffer (pH 6.6) was used. Changes in the pH of the cultures were measured after 14 d incubation. The criterion used for positive substrate fermentation was a pH decrease of at least 0.2. Salt-tolerance tests were carried out with ABCM medium containing NaCl at concentrations ranging from 0 to 25% (w/v). The range of growth temperatures was also determined in ABCM medium. The effect of pH on growth was tested in ABCM medium containing 100 mM MOPS buffer. The pH was adjusted with 4 M NaOH or HCl. Growth was monitored by measuring the increase in OD₆₆₀.

For the analysis of fermentation products from the medium, the strain was incubated in 15 ml complex medium containing 0.5% glucose in 30 ml serum bottles under nitrogen gas. For analysis of broth samples, a Shimadzu organic acid analysis system using ion-exclusion chromatography was employed. Ethanol in broth samples was analysed with enzymic kits from Boehringer Mannheim. The gas phase was analysed by a Hitachi G-5000A gas chromatograph

equipped with a thermal-conductivity detector. The effect of external electron acceptors on growth was tested in basal medium containing glucose (10 mM) as an electron donor. Compounds tested as electron acceptors included elemental sulfur, sodium sulfite, sodium sulfate, thiosulfate and sodium formate at a concentration of 3 g l⁻¹.

G + C content of genomic DNA and DNA–DNA hybridization.

Genomic DNA was prepared by the procedure of Marmur (1961). DNA–DNA hybridization studies were carried out by the microplate hybridization method with photobiotin labelling and fluorometric detection as described previously (Ezaki *et al.*, 1989). The DNA base composition was determined by the HPLC method as reported previously (Katayama-Fujimura *et al.*, 1984).

16S rRNA gene sequencing and phylogenetic analysis. The 16S rRNA gene was amplified by PCR. Sequencing of the 16S rRNA gene was carried out by using an Applied Biosystems PCR kit (Dye Terminator cycle sequencing kit). For amplification of the 16S rRNA gene, a universal primer set was used that corresponded to positions 8–27 (forward primer) and 1492–1510 (reverse primer) of the *Escherichia coli* numbering system (Weisburg *et al.*, 1991). The reaction mixture was analysed with the Applied Biosystems 373A DNA sequencer as described previously (Satomi *et al.*, 1997). The final sequence was determined from overlapping sequence data using the GENETYX computer program.

Nucleotide substitution rates (*K*_{unc} values) (Kimura, 1980) were determined and a distance matrix tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) using the CLUSTAL W program (Thompson *et al.*, 1994). The sequence for positions 99–1359 in the *E. coli* numbering was used in the analyses.

Reference sequences of other members of the genus *Haloanaerobium* and related genera were obtained from the DDBJ database. The following sequences were used for analysis (with accession numbers): *Haloanaerobium praevalens* DSM 2228^T (AB022034), *Haloanaerobium acetoethylicum* DSM 3532^T (X89071), *Haloanaerobium saccharolyticum* subsp. *saccharolyticum* DSM 6643^T (X89069), *Haloanaerobium saccharolyticum* subsp. *senegalense* DSM 7379^T (X89070), *Haloanaerobium alcaliphilum* DSM 8275^T (X81850), *Haloanaerobium lacusrosei* DSM 10165^T (L39787), *Haloanaerobium congolense* DSM 11287^T (U76632), *Haloanaerobium salsuginis* ATCC 51327^T (L22890), *Haloanaerobium kushneri* ATCC 700103^T (U86446), *Halobacteroides halobius* DSM 5150^T (L37423), *Sporohalobacter lortetii* ATCC 35059^T (M59122), *Halo-thermothrix orenii* OMC 544^T (L22016) and *Megasphaera elsdenii* ATCC 17752 (M26493).

RESULTS

Morphology and cultural characteristics

Cells of isolate R-9^T were Gram-negative rods (1.0–1.2 µm wide by 2.7–3.3 µm long). Cells grew only anaerobically on plate medium. Colonies on ABCM plate medium supplemented with 10% (w/v) NaCl were smooth, convex, opaque and yellowish. Spores were not observed microscopically and no growth was obtained after pasteurization. Strain R-9^T was motile by means of peritrichous flagella (Fig. 1).

Fig. 1. Transmission electron micrograph of negatively stained cells of *Haloanaerobium fermentans* strain R-9^T. Bar, 2 µm.

Physiological and biochemical characteristics

Isolate R-9^T was not able to grow in the presence of less than 5 % (w/v) NaCl and growth was not inhibited in the presence of 25 % (w/v) NaCl. The optimal NaCl concentration for growth was 10 % (w/v). Isolate R-9^T grew well at 15 and 45 °C but not at 10 or 50 °C. The optimum temperature for growth was 35 °C. It grew well in the pH range of 6.0 to 9.0. The optimum pH for growth was 7.5. It grew in an anaerobic box with a GasPak anaerobic system (BBL) but not outside the anaerobic box. Isolate R-9^T fermented D-glucose to hydrogen, carbon dioxide and other acidic end-products. It produced acetate, formate, lactate and ethanol, but not butyrate or propionate. After 6 d incubation, isolate R-9^T had produced 258 mg acetic acid l⁻¹, 20 mg formic acid l⁻¹, 41 mg lactic acid l⁻¹ and 603 mg ethanol l⁻¹ in the broth culture. Isolate R-9^T produced hydrogen sulfide on SIM medium. Fer-

Table 1. Differentiation of isolate R-9^T from other *Haloanaerobium* species on the basis of biochemical and physiological characteristics

H., *Haloanaerobium*; NT, not tested; ±, slight growth. Data for other species were taken from Zeikus *et al.* (1983) (*H. praevalens*), Rengpipat *et al.* (1988) (*H. acetothyliticum*), Zhilina *et al.* (1992) (*H. saccharolyticum* subsp. *saccharolyticum*), Cayol *et al.* (1994) (*H. saccharolyticum* subsp. *senegalense*), Tsai *et al.* (1995) (*H. alcaliphilum*), Cayol *et al.* (1995) (*H. lacusrosei*), Ravot *et al.* (1997) (*H. congolense*), Bhupathiraju *et al.* (1994) (*H. salsuginis*) and Bhupathiraju *et al.* (1999) (*H. kushneri*).

Characteristic	R-9 ^T	<i>H. praevalens</i> DSM 2228 ^T	<i>H. acetothyliticum</i> DSM 3532 ^T	<i>H. saccharolyticum</i> subsp. <i>saccharolyticum</i> DSM 6643 ^T	<i>H. saccharolyticum</i> subsp. <i>senegalense</i> DSM 7379 ^T	<i>H. alcaliphilum</i> DSM 8275 ^T	<i>H. lacusrosei</i> DSM 10165 ^T	<i>H. congolense</i> DSM 11287 ^T	<i>H. salsuginis</i> ATCC 51327 ^T	<i>H. kushneri</i> ATCC 700103 ^T
Cell size (µm)	1.1–2 × 2.7–3.3	0.9–1.1 × 2–2.6	0.4–0.7 × 1–1.6	0.5–0.7 × 1–1.5	0.4–0.6 × 2–5	0.8 × 3.3–5	0.5 × 2–3	0.5–1 × 2–4	0.3–0.4 × 2.6–4	0.7 × 2–3.3
Motility	+	–	+	+	+	+	+	–	–	+
G + C content (mol %)	33	27	32	31	32	31	32	34	34	34
NaCl concentration (%)										
Range	7–25	2.0–30	6–20	3–30	5–25	2.5–25	7.5–34	4–24	6–24	9–18
Optimum	10	12.5	10	10	7.5–12.5	10	18–20	10	9	12
Temperature (°C):										
Range	15–45	5–50	15–45	15–47	20–47	25–50	20–50	20–45	22–51	20–45
Optimum	35	37	34	37–40	40	37–40	40	42	40	40
pH:										
Range	6.0–9.0	6.0–9.0	5.4–8.0	6.0–8.0	6.3–8.7	5.8–10	NT	6.3–8.5	5.6–8.0	6.0–8.0
Optimum	7.5	7.0–7.4	6.3–7.4	7.5	7.0	6.7–7.0	7.0	7.0	6.1	6.5–7.5
Utilization of:										
L-Arabinose	–	NT	NT	+	–	–	–	–	+	+
N-Acetylglucosamine	+	+	+	+	NT	+	NT	NT	+	NT
Cellobiose	+	–	+	+	+	–	+	NT	–	+
Fructose	+	+	+	+	+	+	+	+	+	+
Galactose	+	–	–	+	–	–	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+
Glycerol	–	–	–	+	+	–	+	NT	–	–
Lactose	+	–	+	+	+	–	+	–	+	+
Maltose	+	NT	NT	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	+	+
Pyruvate	–	–	+	+	NT	+	NT	NT	+	+
Raffinose	+	NT	NT	–	+	±	NT	NT	+	NT
D-Ribose	+	NT	NT	+	+	–	+	+	+	NT
Rhamnose	–	NT	NT	–	–	–	–	–	+	NT
Starch	–	–	–	NT	NT	–	+	NT	–	–
Sucrose	+	–	+	+	+	+	+	+	+	+
D-Xylose	+	–	+	+	–	–	+	–	+	–
Fermentation products	Acetate, formate, lactate, ethanol, H ₂ , CO ₂	Acetate, butyrate, propionate, H ₂ , CO ₂	Acetate, ethanol, H ₂ , CO ₂	Acetate, H ₂ , CO ₂	Acetate, H ₂ , CO ₂	Acetate, lactate, butyrate, H ₂ , CO ₂	Acetate, ethanol, H ₂ , CO ₂	Acetate, H ₂ , CO ₂	Acetate, ethanol, H ₂ , CO ₂	Acetate, formate, ethanol, H ₂ , CO ₂

Table 2. Degree of DNA–DNA relatedness between the new isolate and other *Haloanaerobium* species

Taxon	Strain	Relatedness to labelled DNA from R-9 ^T (%)
New isolate	R-9 ^T (= JCM 10494 ^T)	100
<i>H. praevalens</i>	DSM 2228 ^T	22
<i>H. acetoethylicum</i>	DSM 3532 ^T	23
<i>H. saccharolyticum</i> subsp. <i>saccharolyticum</i>	DSM 6643 ^T	20
<i>H. saccharolyticum</i> subsp. <i>senegalense</i>	DSM 7379 ^T	11
<i>H. alcaliphilum</i>	DSM 8275 ^T	20
<i>H. lacusrosei</i>	DSM 10165 ^T	19
<i>H. congolense</i>	DSM 11287 ^T	21
<i>H. salsuginis</i>	ATCC 51327 ^T	11

H., *Haloanaerobium*.

Fig. 2. Phylogenetic tree showing the relationship of *Haloanaerobium fermentans* strain R-9^T (= JCM 10494^T) to other strains of the genus *Haloanaerobium* and related genera based on a comparison of 16S rRNA gene sequences. The tree, constructed by the neighbour-joining method (Saitou & Nei, 1987), was based on a comparison of positions 99–1359 (*E. coli* numbering; Weisburg et al., 1991). The tree was rooted with *Megasphaera elsdenii*.

mentative substrates included *N*-acetylglucosamine, cellobiose, fructose, galactose, D-glucose, lactose, maltose, D-mannose, raffinose, D-ribose, sucrose and D-xylose. L-Arabinose, glycerol, pyruvate, rhamnose and starch were not fermented (Table 1). Sulfate, sulfite, thiosulfate, elemental sulfur and formate did not stimulate the growth of isolate R-9^T.

G + C content of DNA and DNA–DNA hybridization

The DNA base composition of isolate R-9^T was found to be 33.3 mol% G + C. The levels of DNA hybridization between the new isolate and seven *Haloanaerobium* species used in this study were less than 30% (Table 2).

16S rRNA gene sequence comparison

In order to investigate the phylogenetic relationship of isolate R-9^T to other bacteria, the sequence of a 16S rRNA gene PCR product was determined and then analysed. A comparative analysis of the 16S rRNA gene sequences revealed that isolate R-9^T belonged to the low-G + C-content, Gram-positive phylum. Further sequence alignments and phylogenetic analyses show the taxonomic and phylogenetic position of the new isolate to be among the members of the genus *Haloanaerobium* (Fig. 2). The sequence similarities of isolate R-9^T to other *Haloanaerobium* species ranged from 95.1% (*Haloanaerobium salsuginis*) to 97.7% (*Haloanaerobium saccharolyticum* subsp. *saccharolyticum*).

DISCUSSION

The strain that we investigated is a strictly anaerobic, rod-shaped, fermentative bacterium that is Gram-negative, non-spore-forming and motile by means of peritrichous flagella. It is catalase-negative, moderately halophilic and has a genomic DNA G + C content of 33 mol%. On the basis of these characteristics, the isolate should be considered a member of the order *Haloanaerobiales* (Rainey *et al.*, 1995).

The phylogenetic analyses of 16S rRNA gene sequence information clarify the taxonomic and phylogenetic position of isolate R-9^T in the genus *Haloanaerobium*. Isolate R-9^T could not be accommodated in the same species as any of the seven *Haloanaerobium* species described previously due to the low DNA–DNA hybridization values (Table 2).

Phenotypic studies also revealed that isolate R-9^T could not be assigned to any of the previously described species of the genus *Haloanaerobium* (Table 1). Isolate R-9^T differs from *Haloanaerobium praevalens* and *Haloanaerobium alcaliphilum* in its ability to ferment a wide range of carbohydrates and in its fermentation products (volatile organic acids and ethanol). In addition, isolate R-9^T and *Haloanaerobium praevalens* can be differentiated by mo-

tility. *Haloanaerobium lacusrosei* (Cayol *et al.*, 1995) is an extremely halophilic bacterium that grows optimally in 18–20% (w/v) NaCl. Therefore, isolate R-9^T could be differentiated from *Haloanaerobium lacusrosei* on the basis of optimal salt concentration, since optimal growth of isolate R-9^T was observed in 10% (w/v) NaCl, as well as some differences of substrate profiles, such as the ability to ferment glycerol and starch. Isolate R-9^T can be differentiated from *Haloanaerobium saccharolyticum* (both subspecies) (Zhilina *et al.*, 1992; Cayol *et al.*, 1994) on the basis of its ability to produce ethanol. In addition, the ability to ferment L-arabinose, glycerol, pyruvate and raffinose can be used to differentiate isolate R-9^T from *Haloanaerobium saccharolyticum* subsp. *saccharolyticum*. Likewise, the ability to ferment galactose, glycerol and D-xylose can be used to differentiate it from *Haloanaerobium saccharolyticum* subsp. *senegalense*. Other combinations of properties distinguish isolate R-9^T from the three other *Haloanaerobium* species: from *Haloanaerobium acetoethylicum* (Rengpipat *et al.*, 1988) by fermentation of galactose and pyruvate; from *Haloanaerobium salsuginis* (Bhupathiraju *et al.*, 1994) by fermentation of L-arabinose, cellobiose, pyruvate and rhamnose and by motility; and from *Haloanaerobium congolense* (Ravot *et al.*, 1997) by fermentation of lactose and D-xylose and by motility.

Recently, *Haloanaerobium kushneri* was isolated from an oil brine (Bhupathiraju *et al.*, 1999). Since *Haloanaerobium kushneri* was not included as a reference in the DNA–DNA hybridization study, we could not compare the genotype of our isolate with that of *Haloanaerobium kushneri*. However, the information available for *Haloanaerobium kushneri* indicates that there are some differences (ability to ferment L-arabinose and some physiological characteristics such as ability to grow at 15 °C or at pH values greater than 8.0) between this species and our isolate. We compared the 16S rRNA gene sequence of isolate R-9^T with the sequence of *Haloanaerobium kushneri* and found a level of similarity of 96.6%. This value is low enough to warrant placement of the two organisms in separate species.

Ecological importance of isolate R-9^T (*Haloanaerobium fermentans*)

Because *Haloanaerobium fermentans* was isolated in the manufacturing process of fermented puffer fish ovaries, its true ecological niche is unknown. However, the presence of this bacterium as one of the dominant species in a salt-preserved food (Kobayashi *et al.*, 1995) indicates the significance of strictly anaerobic, halophilic bacteria during fermentation processes in food products. To our knowledge, there have been few reports of halophilic anaerobes present in food products and associated with the microbiological problems of their manufacturing processes. A halophilic anaerobe, '*Bacteroides halosmophilus*', was isolated from Mediterranean salted anchovies (Baum-

gartner, 1937), but this isolate has been lost. Similar anaerobes were also isolated from a spoiled sample of sugar salted herring (Knøchel & Huss, 1984a, b), but these isolates were not characterized in detail. Recently, we isolated a strictly anaerobic halophile from canned Swedish fermented herrings (Surströmming) and these isolates were identified as *Haloanaerobium praevalens* (unpublished data). Therefore, this report is the first description of a novel halophilic anaerobe isolated from food products. In the manufacturing process of salted puffer fish ovaries, as well as in broth medium, significant amounts of organic acids were found; 30–1570 µg acetic acid g⁻¹, 30–110 µg formic acid g⁻¹ and 310–2050 µg lactic acid g⁻¹ were detected in the fermented ovaries. These halophilic anaerobes probably contribute to the manufacturing process of this Japanese traditional food product, fermented puffer fish ovaries (*fugunoko nukaduke*).

Description of *Haloanaerobium fermentans* sp. nov.

Haloanaerobium fermentans (fer.men'tans. L. part. adj. *fermentans* fermenting).

Cells are Gram-negative rods (1.0–1.2 µm wide by 2.7–3.3 µm long). The bacterium is motile by means of peritrichous flagella. Spores are not observed. Cells grow only anaerobically on solid medium. Colonies on ABCM plate medium supplemented with 10% (w/v) NaCl are smooth, convex, opaque and yellowish. It is a moderately halophilic bacterium that grows in the presence of 7–25% (w/v) NaCl, with an optimal NaCl concentration of 10% (w/v). It grows well at 15 and 45 °C but not at 10 or 50 °C. The optimum temperature for growth is 35 °C. It grows well in the pH range of 6.0 to 9.0 with the optimum pH for growth being 7.5. It ferments D-glucose to hydrogen, carbon dioxide, ethanol and other acidic end-products such as acetate, formate and lactate. Butyrate and propionate are not produced. Hydrogen sulfide is produced. The fermentative substrates include *N*-acetylglucosamine, cellobiose, fructose, galactose, D-glucose, lactose, maltose, D-mannose, raffinose, D-ribose, sucrose and D-xylose. L-Arabinose, glycerol, pyruvate, rhamnose and starch are not fermented. The DNA base composition is 33.3 mol% G + C (HPLC).

Isolated from salted ovaries in the manufacturing process of a Japanese traditional food product, fermented puffer fish ovaries or *fugunoko nukaduke*. The 16S rRNA gene sequence of the new isolate R-9^T has been deposited in the DDBJ database under accession no. AB023308. The type strain is R-9^T, which has been deposited with the Japan Collection of Microorganisms under accession number JCM 10494^T.

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