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TAXONOMIC DESCRIPTION

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Blautia intestinalis sp. nov., isolated from human feces

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Abstract

A strictly anaerobic bacterial strain (27-44^T) was isolated from a stool specimen from an autistic child collected in PR China. The strain was Gram-stain-positive, non-motile, non-pigmented, non-spore-forming, and cells were oval to rod-shaped. Strain 27-44^T grew at 20–40 °C (optimal at 37 °C) and at pH 6.0–10 (optimal at 6.0–8.0). The major polar lipids were one phospholipid, two glycolipids, two aminophospholipids and one unidentified lipid. The major cellular fatty acids of strain 27-44^T were $C_{16:0}$ and $C_{17:0}$ 2-OH. The end product of glucose fermentation was mainly butyric acid. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain 27-44^T was a member of the genus *Blautia* and phylogenetically closely related to *Blautia obeum* ATCC 29174^T (with 97.8% seque nce similarity). The genome of strain 27-44^T was 3.5 Mbp with a DNA G+C content of 42.36 mol%. A total of 3436 genes were predicted and, of these, 3133 genes were annotated by KEGG. On the basis of phenotypic, chemotaxonomic and phylogenetic comparisons, strain 27-44^T represents a novel species within the genus *Blautia*, for which the name *Blautia intestinalis* sp. nov. is proposed. The type strain is 27-44^T= CGMCC 1.5285^T=NBRC 113774^T.

The human gastrointestinal tract harbours diverse microbial communities [1]. Members of *Blautia* occur widely in the gastrointestines of adults and children, and the *Blautia* population in healthy children is more abundant than that in autistic children [2]. The genus *Blautia* was first proposed in 2008 by Liu *et al.* [3]. Several species of *Blautia* were included in the genera *Clostridium* and *Ruminococcus*. Based on the 16S rRNA sequences of these species, they were highly related to each other and formed a subline within the clostridial rRNA cluster XIVa. At the time of writing, the genus *Blautia* comprises 21 species and 15 validly published species with correct names (www.bacterio.net/Blautia.html). The validly published names are *Blautia argi* [4], *Blautia caecimuris* [5], *Blautia coccoides* [3, 6], *Blautia faecicola* [7], *Blautia faecis* [8], *Blautia glucerasea* [9], *Blautia hansenii* [3, 10], *Blautia hominis* [11], *Blautia hydrogentrophica* [3, 12], *Blautia luti* [3, 13], *Blautia obeum* [14, 15], *Blautia producta* [3, 10], *Blautia schinkii* [3, 16], *Blautia sterocoris* [17] and *Blautia wexlerae* [3]. *B. coccoides* is the type species of the genus. Cells of the members of *Blautia* are Gram-positive, non-motile and coccoid or oval-shaped. The major end-products of glucose fermentation are acetate, ethanol, hydrogen, lactate and succinate. The G+C contents are 35–47 mol%. During our study of the microbial diversity in a faecal sample of an autistic patient, bacterial isolate 27-44^T was obtained. Here we report the characterization and taxonomy of this bacterial isolate 27-44^T.

Stool samples were frozen by liquid nitrogen immediately after collection, shipped on dry ice, and stored in −80 °C freezer. Samples were processed in an anerobic chamber (Elecotrek) filled with 85% N_2, 10% H_2 and 5% CO_2. Washed thrice with PBS, the suspension was filtered through a 40 µm cell strainer (Falcon), serially diluted in PBS and spread on modified yeast extract–casitone–fatty acid (YCFA) medium with following composition (per litre distilled water): 10.0 g casitone, 2.5 g yeast extract, 2.0 g glucose, 0.45 g KH_2PO_4, 0.45 g K_2HPO_4, 4.0 g $NaHCO_3$, 0.9 g NaCl, 0.01 g $MgSO_4{\cdot}7H_2O$, 0.09 g $CaCl_2{\cdot}2H_2O$, 0.01 g haemin (dissolved in NaOH solution and adjusted to pH 7), 1.0 g L-cysteine, 1 ml resazurin (0.1%). The pH was adjusted to pH 7.0 with 1M NaOH solution.

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Keywords: *Blautia intestinalis*; faeces; human intestinal microbiota; strain 27-44^T.
Abbreviations: ANI, average nucleotide identity; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; YCFA, yeast extract–casitone–fatty acid.
The 16S rRNA gene sequence of strain 27-44^T is deposited at GenBank/EMBL/DDBJ under accession number MK224720 and NMDC under accession number NMDCN000011P. The genomic data of 27-44^T is deposited at DDBJ/ENA/GenBank under accession number JACOQE000000000 and at NMDC under accession number NMDC60014055.
Four supplementary figures are available with the online version of this article.

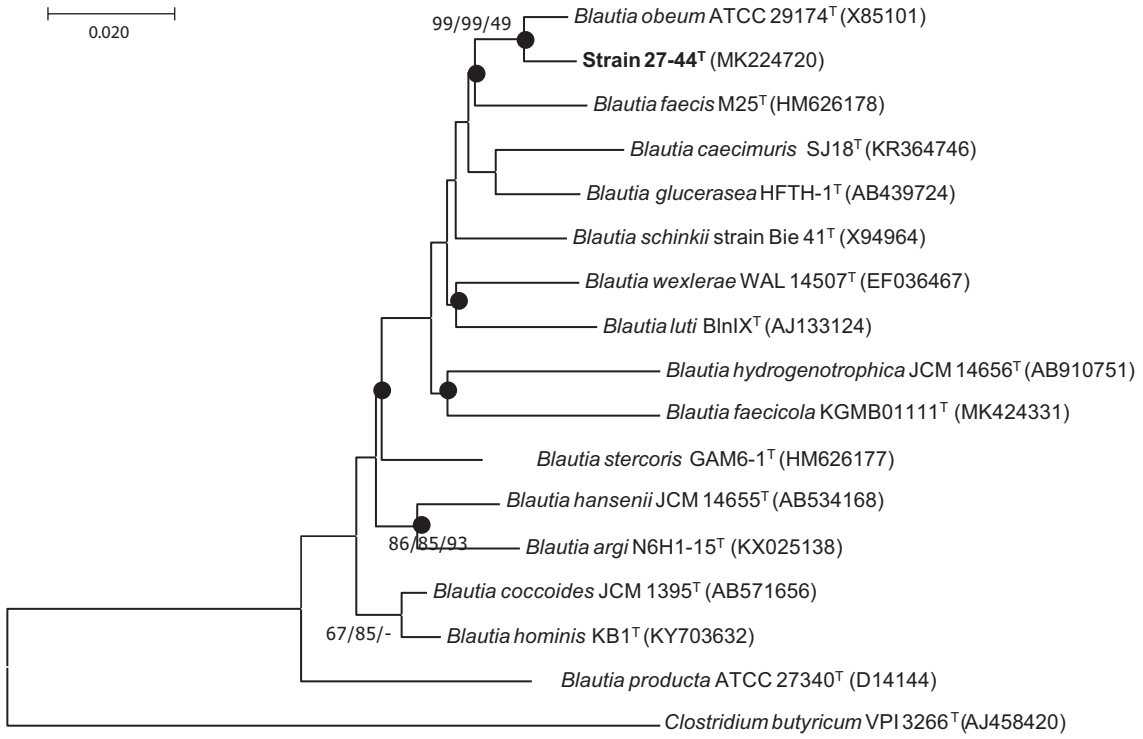

Fig. 1. Phylogenetic tree reconstructed with the neighbour-joining method based on 16S rRNA sequences of strain 27-44 ^{T} and other species within the genus *Blautia*. The type species *Clostridium butyricum* VPI13266^T was used as an outgroup. Sequences were downloaded from NCBI GenBank. Filled circles indicate branches that are found in trees generated with all three methods: neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP). Numbers at branch nodes represent confidence levels (values >70% are shown) from 1000 replicate bootstrap samplings, followed by the numbers of NJ/ML/MP. GenBank accession numbers are given in parentheses. Bar, 0.02 substitutions per nucleotide position.

The solid medium of modified YCFA contained 1.5% agar. The inoculated agar plates were cultivated for 3 days at 37 °C under anaerobic conditions. Colonies were picked and repeatedly streaked on modified agar plates until pure culture was obtained. The isolate was preserved in 10% skimmed milk and stored at −80 °C.

The 16S rRNA gene was amplified by PCR using the 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACG GCTACCTTGTTACGACTT-3′) primers [18]. Comparative analysis on BLAST (GenBank/EMBL/DDBJ database) revealed that the 16S rRNA gene sequence (1344 bp, GenBank accession no. MK224720) of strain 27-44 ^{T} was closely related to that of *B. obeum* ATCC 29174^T (97.8% similarity), which indicated that strain 27-44 ^{T} was a member of the genus *Blautia*. Phylogenetic trees were reconstructed based on 16S rRNA gene sequences by MEGA version 7.0.26 [19, 20] using the neighbour-joining (NJ) [21], maximum-likelihood (ML) [22] and maximum-parsimony (MP) [23] methods. The NJ tree was generated based on Kimura's two-parameter method [24]. Branching patterns of trees were evaluated by bootstrapping with 1000 replications [25]. The ML tree was built based on the best-fit model 'K2 +G+I'. The bootstrap consensus trees resulted from ML and MP (Figs S1 and S2, available in the online version of this article) supported the NJ tree (Fig. 1).

The phylogenetic tree revealed that strain 27-44^T was clustered within a branch comprising other species belonging to the genus *Blautia* and closely related to *B. obeum* ATCC 29174^T.

Genomic DNA was extracted from cultures grown in liquid modified YCFA medium by using Wizard genome DNA Purification Kit (Promega, A1120). The genome of strain 27-44^T was sequenced using the HiSeq X-Ten platform (Illumina). Pair-end sequencing (2×150 bp) of a fragment library created about 1×10^7 quality-filtered reads. Filtered reads assembled into 44 scaffolds with a total sequence length of 3481978 bp (contig N50: 391748 bp). The G+C content of the genome of strain 27-44^T was 42.36 mol%, which was within the range of the genus *Blautia*. Calculated using the average nucleotide identity (ANI) calculator from EzBioCloud [26], the ANI value between 27-44^T and its closest species *B. obeum* ATCC 29174^T was 83.45%. Analysed by the Genome-to-Genome Distance Calculator 2.1 [27], the digital DNA–DNA hybridization estimation between 27-44^T and *B. obeum* ATCC 29174^T is 27.50%. Using Glimmer3 [28], 3436 genes was predicted. The obtained genome sequence data was subsequently annotated using the NCBI-NR, Swiss-Port, KEGG, COG, GO and CAZy databases. Based on the KEGG annotation, there were 178 genes related to carbohydrate metabolism, 141 genes related to amino acid metabolism, 114 genes related

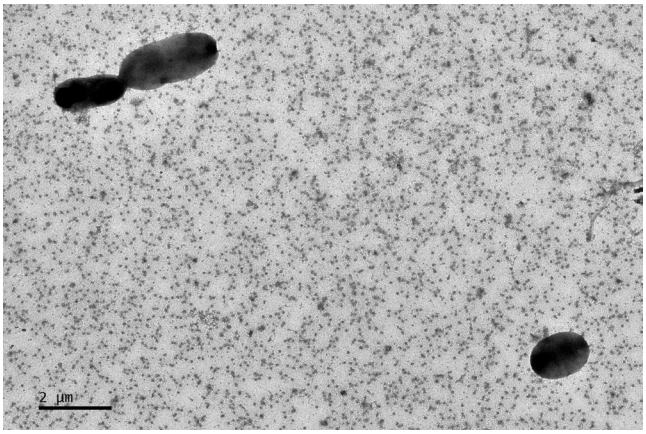

Fig. 2. Transmission electron microscope image of strain 27–44^T cells grown on modified YCFA agar. Bar: 2 µm.

to cofactors and vitamins and 82 genes related to nucleotide metabolism.

After cultivation on modified YCFA agar plates for 2 days at 37 °C, cells were harvested and stained with sodium phosphotungstate. Cell morphology was observed with transmission electron microscopy (JEM-1400, JEOL). Gram staining was carried out based on the standard method [29] and the result was verified by KOH solution lysis test [30]. Phase-contrast microscopy was used to check the presence of spores on culture. The temperature range for growth was determined at 4, 16, 20, 30, 37, 40, 45, and 60 °C after incubation for 3 days. The pH range for growth was tested at pH 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0 and 11.0 after incubation for 5 days at 37 °C, by adjusting the pH prior to sterilization with 0.1 M sodium acetate buffer (for pH 4.0–6.0), 0.1 M phosphate buffer (for pH 7.0–8.0), Tris buffer (for pH 8.0–9.0) or 1.0 M NaOH solution (for pH 10.0–11.0). NaCl tolerance was determined in modified YCFA medium supplemented with 0–5% (w/v) NaCl at intervals of 0.5%. Cell growth was estimated by measuring turbidity at 600 nm (OD_{600}) using a UV/visible spectrophotometer (SPECORD205, Analytic Jena). Aerobic growth was tested using modified YCFA medium and incubated at 37 °C for 3 days.

Table 1. Cellular fatty acid profiles (% of totals) of strain 27-44^T and *Blautia obeum* ATCC 29174^T

Strains: 1, 27-44^T; 2, ATCC 29174^T. Fatty acids that represent <1% of the total in all strains are not shown. All data are from this study. Major components are indicated with bold text.

Fatty acid	1	2
$C_{14:0}$	7.8	5.7
$C_{15:1}$ $\omega 8c$	5.5	5.9
$C_{16:1}$ $\omega 5c$	1.4	–
$C_{16:0}$	**38.0**	**29.9**
anteiso-$C_{17:1}$ $\omega 9c$	3.5	4.3
$C_{17:1}$ $\omega 7c$	6.6	9.1
$C_{18:1}$ $\omega 9c$	1.8	1.9
$C_{18:0}$	2.9	10.6
$C_{17:0}$ 2-OH	**17.1**	**21.3**
Summed feature 3*	6.5	2.4
Summed feature 8*	8.1	7.6

*Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. Summed feature 3 consists of $C_{16:1}$ $\omega 7c$/$C_{16:1}$ $\omega 6c$ and/or $C_{16:1}$ $\omega 6c$/$C_{16:1}$ $\omega 7c$; summed feature 8 consists of $C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$.

Cell morphology was observed after 2 days of incubation on modified YCFA agar media at 37 °C. Colonies of strain 27-44^T were whitish, circular, entire and umbonate with diameter of 0.8–2 mm. Observed under TEM, cells were oval rod-shaped, 2.1–2.8 µm long, 1.0–1.2 µm wide and appeared singly or in pairs based on growth phase (Fig. 2). Cell motility was examined using light microscopy (Axiostar plus, Zeiss). Cells were Gram-stain-positive and non-motile.

Strain 27-44^T grew at 20–40 °C (optimal at 37 °C), and no growth was observed at <15 °C or >45 °C. No spores were observed. Strain 27-44^T grew at pH 6.0–10.0 (optimal at pH 6.5–7.5) and no growth was observed at pH 5.0 or pH 11.0. Strain 27-44^T grew with 0–1% (w/v) NaCl and no growth occurred with 1.5% NaCl. No growth was observed in aerobic conditions.

Biochemical characteristics were determined using the API 20A anaerobe test system (bioMérieux), the AN MicroPlate (biolog), the Rapid ID 32A anaerobe identification system and the API ZYM system (both bioMérieux) according to the manufacturers' instructions. In API 20A tests, strain 27-44^T could be differentiated from *B. obeum* ATCC 29174^T by its ability to ferment glucose, lactose, maltose, salicin, xylose, arabinose, cellobiose, mannose, raffinose, sorbitol and rhamnose. Strain 27-44^T could hydrolyse aesculin, but not gelatin. In the AN MicroPlate, strain 27-44^T could use *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, amygdalin, arbutin, cellobiose, dextrin, D-galacturonic acid, dulcitol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, glucose-1-phosphate, glucose-6-phosphate, lactose, lactulose, maltose, maltotriose, D-mannose, melezitose, melibiose, 3-melthyl-D-glucose, methyl α-D-galactoside, palatinose, methyl β-D-galactoside, α-methyl-D-glucoside, methyl β-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol, stachyose, sucrose, trehalose, turanose, glyoxylic acid, α-hydroxybutyric acid, L-serine, L-threonine, inosine and thymidine-5′-monophosphate, and weakly utilized 3-melthyl-D-glucose and m-inositol. For enzyme activities, strain 27-44^T could be differentiated from *B. obeum* ATCC 29174^T by its β-galactosidase-6-phosphate, serine arylamidase and alkaline phosphatase activities in the API Rapid ID 32A test. According to API ZYM tests, strain 27-44^T could be

Table 2. Differential characteristics of *Blautia intestinalis* sp. nov. and all other 15 validly published members of the genus *Blautia*

Strains: 1, 27-44^T; 2, *B. obeum* ATCC 29174^T; 3, *B. argi* N6H1-15^T; 4, *B. caecimuris* SJ18^T; 5, *B. coccoides* JCM 1395^T; 6, *B. faecicola* KGMB01111^T; 7, *B. faecis* M25^T; 8, *B. glucerasea* HFTH-1^T; 9, *B. hansenii* JCM 14655^T; 10, *B. hominis* KB1^T; 11, *B. hydrogentrophica* JCM 146563^T; 12, *B. luti* bln9^T; 13, *B. producta* ATCC 27340^T; 14, *B. schinkii* Bie 41^T; 15, *B. sterocoris* GAM6-1^T; 16, *B. wexlerae* WAL 14507^T. Data of 1 and 2 were from this study and other data were from previous studies [3–5, 7–9, 11, 13, 17]. +, Positive; –, negative; w, weakly positive; d, different among strains; ND, not detected.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
End products of metabolism*	P, B, A	A	A, L	ND	A, S	A	L, A	A, F, L	L, A	A, S, L, F	A	A, S	L, A	A	A	A, S
Fermentation of:																
Arabinose	+	+	+	+	+	w	+	+	–	+	–	+	+	+	+	+
Cellobiose	+	+	+	–	+	–	–	+	–	+	+	+	+	+	+	d
Lactose	+	+	+	+	+	–	–	+	+	+	+	+	+	+	–	d
Mannose	–	+	–	–	+	–	–	–	–	+	+	+	+	+	–	+
Maltose	+	+	+	+	+	–	–	+	+	+	+	+	+	+	–	d
Raffinose	+	+	–	+	+	+	–	+	+	+	+	+	+	+	–	d
Sucrose	–	–	+	+	+	w	–	–	–	+	–	+	–	–	–	d
Xylose	+	+	–	+	+	–	–	+	–	–	ND	+	+	+	–	+
α-Galactosidase	+	+	+	+	+	+	+	+	–	+	–	ND	+	+	+	+
β-Galactosidase	+	+	+	+	+	+	+	+	+	+	–	ND	+	w	+	+
α-Glucosidase	+	+	+	+	ND	+	+	–	–	+	ND	ND	+	w	+	+
β-Glucosidase	+	+	+	–	–	+	+	+	–	+	–	ND	w	+	+	+
α-Arabinosidase	–	–	+	–	+	+	+	–	–	+	+	+	+	+	+	+
Esterase (C4)	+	–	–	ND	ND	+	–	–	–	ND	–	ND	–	–	–	d
Acid phosphatase	+	+	–	ND	ND	+	–	+	–	ND	+	ND	+	–	–	+
Alkaline phosphatase	+	+	–	ND	–	–	w	–	–	+	–	+	–	–	–	–
N-Acetyl-β-glucosaminidase	–	–	–	ND	+	+	–	+	+	+	–	+	–	–	–	–
DNA G+C content (mol%)	42	40–41	44	ND	44	45	42	41	44–45	46	45	43	44–45	46–47	36	ND
Source	Human faeces	Human faeces	Human faeces	Mouse faeces	Mouse faeces	Human faeces	Human faeces	Dog faeces	Human faeces	Human faeces	Human faeces	Human faeces	Human faeces	Human faeces	Human faeces	Human faeces

*A, acetic acid; B, butyric acid; F, formic acid; L, lactic acid; P, propionic acid; S, succinic acid.

differentiated from *B. obeum* ATCC 29174^T by being positive for esterase (C4) and β-glucosidase, and lack of activity of alkaline phosphatase or α-glucosidase.

Short-chain fatty acid analysis was undertaken using a culture of strain 27-44^T incubated in modified YCFA medium at 37 °C for 12 h. For testing acetic acid, propionic acid, butyric acid, pentanoic acid and valeric acid production, 1 ml whole culture of strain 27-44^T was extracted in 1 ml ethyl acetate. The supernatant liquid was prepared for GC-MS analysis. GC-MS analysis was performed on a GCMS-QP2010 Ultra with an auto sampler (Shimadzu) and a DB-wax capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness; Shimadzu). The temperature of the oven was programmed from 80 to 140 °C at 20 °C min^{-1} gradient, with a 1 min hold; to 290 °C at 3.5 °C min^{-1}, with a 15 min hold. Injection of a 1 µl sample was performed at 280 °C. The carrier gas, helium, flowed at 1.2 ml min^{-1}. Electronic impact was recorded at 70 eV [31]. Strain 27-44^T produced mainly propionic acid and butyric acid, and lesser amounts of valeric acid and acetic acid when grown in modified YCFA broth.

Polar lipids were extracted from 0.1 g lyophilized cells, separated by two-dimensional TLC (plates coated with silica gel, 10×10 cm; Merck). Chromatography was performed using chloroform–methanol–water (65:25:4, v/v/v) for the first dimension, followed by chloroform–methanol–acetic acid–water (80:12:15:4, v/v/v/v) for the second dimension. The plates were developed by spraying with appropriate detection reagents [32]. Total lipids were stained with 10% ethanolic molybdatophosphoric acid (Sigma), aminolipids were detected with 0.4% solution of ninhydrin (Sigma) in butanol, phospholipids were detected with Zinzadze reagent (molybdenum blue spray reagent, 1.3%; Sigma) and glycolipids were detected with 0.5% α-naphthol reagent. Strain 27-44^T displayed a polar lipid profile consisting of one phospholipid, two glycolipids, two aminophospholipids and one unidentified lipid was observed (Fig. S3).

The cellular fatty acid profiles were determined based on the standard protocol of the Sherlock Microbial Identification System version 6.0 (MIDI). Fatty acids were harvested, saponified and methylated. The components were analysed by gas chromatography (HP 6890, Agilent) and identified using the TSBA6 database of the Microbial Identification System [33]. The major cellular fatty acids (>10% total fatty acids) of strain 27-44^T were revealed to be $C_{16:0}$ and $C_{17:0}$ 2-OH, which is consistent with *B. obeum* ATCC 29174^T. Although the predominant fatty acids resembled those of the reference, small differences in minor components were observed. The fatty acid profiles of strain 27-44^T and *B. obeum* ATCC 29174^T are summarized in Table 1.

Based on its phenotypic, physiological, biochemical and phylogenetic characteristics, as well as genomic data, we concluded that strain 27-44^T represents a novel species of the genus *Blautia*, for which the name *Blautia intestinalis* sp. nov. is proposed. The features that differentiate *B. intestinalis* from other members of the genus *Blautia* are listed in Table 2.

DESCRIPTION OF *BLAUTIA INTESTINALIS* SP. NOV.

Blautia intestinalis (N.L. masc. adj. *intestinalis* pertaining to the intestine.)

Cells are Gram-stain-positive, non-pigmented, non-spore-forming, non-motile and oval rod shaped (2.1–2.8 µm long and 1.0–1.2 µm wide). Whitish, circular, entire and umbonate colonies with a diameter of 0.8–2.0 mm appear on modified YCFA agar plates after 2 days of incubation. Growth occurs anaerobically at 20–40 °C (optimal at 37 °C) and pH 6.0–10.0 (optimal at 6.5–7.5), and the strain tolerates 0–1.0% (w/v) NaCl. Indole and urease are not produced. Aesculin is hydrolysed, but not gelatin. The following compounds are utilized for growth: *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, amygdalin, arbutin, cellobiose, dextrin, D-galacturonic acid, dulcitol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, glucose-1-phosphate, glucose-6-phosphate, lactose, lactulose, maltose, maltotriose, D-mannose, melezitose, melibiose, 3-methyl-D-glucose, methyl α-D-galactoside, palatinose, methyl β-D-galactoside, methyl α-D-glucoside, methyl β-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol, stachyose, sucrose, trehalose, turanose, glyoxylic acid, α-hydroxybutyric acid, L-serine, L-threonine, inosine and thymidine-5-monophosphate. Positive reactions in the Rapid ID 32A and API ZYM systems are obtained for α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-galactosidase-6-phosphate, alkaline phosphatase, serine arylamidase and acid phosphatase, while negative reactions are obtained for α-arabinosidase, *N*-acetyl-β-glucosaminidase, serine arylamidase, glutamic acid decarboxylase, α-fucosidase, arginine arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutaml glutamic acid arylamidase, valine arylamidase, α-chymotrypsin, urease, arginine dihydrolase, proline arylamidase and pyroglutamix acid arylamidase. The polar lipid profile includes one phospholipid, two glycolipids, two aminophospholipids and one unidentified lipid. The major fatty acids are $C_{16:0}$ and $C_{17:0}$ 2-OH.

The type strain, 27-44^T (=CGMCC 1.5285^T=NBRC 113774^T), was isolated from a stool sample of an autistic child collected at Peking Union Medical College Hospital. The genomic DNA G+C content of the type strain is 42.36 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement

This study was approved by the Ethics Review Committee of Peking Union Medical College Hospital of Chinese Academy of Medical Sciences (ZS-1393).

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