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TAXONOMIC DESCRIPTION
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Tannockella kyphosi gen. nov., sp. nov., a member of the family *Erysipelotrichaceae*, isolated from the hindgut of the marine herbivorous fish *Kyphosus sydneyanus*

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Abstract

A Gram-stain-positive, non-spore-forming, rod-shaped, obligately anaerobic bacterium, designated strain BP52G^T, was isolated from the hindgut of a Silver Drummer (*Kyphosus sydneyanus*) fish collected from the Hauraki Gulf, New Zealand. Phylogenetic analysis based on 16S rRNA gene sequencing indicated that the isolate belonged to the family *Erysipelotrichaceae* in the phylum Firmicutes and was most closely related to *Clostridium saccharogumia* with 93.3% sequence identity. Isolate BP52G^T grew on agar medium containing mannitol as the sole carbon source. White, opaque and shiny colonies of the isolate measuring approximately 1 mm diameter grew within a week at 20–28 °C (optimum, 24 °C) and pH 6.9–8.5 (optimum, pH 7.8). BP52G^T tolerated the addition of up to 1% NaCl to the medium. Formate and acetate were the major fermentation products. The major cellular fatty acids were $C_{16:0}$, $C_{16:1n-7t}$ and $C_{18:1n-7t}$. The genome sequence of the isolate was determined. Its G+C content was 30.7 mol%, and the 72.65% average nucleotide identity of the BP52G^T genome to its closest neighbour with a completely sequenced genome (*Erysipelatoclostridium ramosum* JCM 1298^T) indicated low genomic relatedness. Based on the phenotypic and taxonomic characteristics observed in this study, a novel genus and species *Tannockella kyphosi* gen. nov., sp. nov. is proposed for isolate BP52G^T (=NZRM 4757^T=JCM 34692^T).

INTRODUCTION

The family *Erysipelotrichaceae* was established in 2004, with *Erysipelothrix* as the type genus [1]. Although the name was derived from the first identified member of the family, *Erysipelothrix rhusiopathiae* [2, 3], which caused erysipelas in pigs, numerous members have since been isolated from the gut of healthy mammals and insects [4–7]. Studies in mice suggest that gut-associated members of the *Erysipelotrichaceae* contribute to host lipid metabolism [8, 9]. *Erysipelotrichaceae* have also been reported in several fish gut microbiota studies [10–12]. *Erysipelotrichaceae* members are characteristically Gram-stain-positive, filamentous or rod-shaped cells that have a unique peptidoglycan type and a low G+C content (~36–40 mol%). Initially this family was described as containing only aerobic to facultatively anaerobic bacteria [1], but this was later amended to include strict anaerobes [7]. At the time of writing, the family consists of the following 25 valid genera: *Absicoccus*, *Absiella*, *Allobaculum*, *Amedibacillus*, *Amedibacterium*, *Anaerorhabdus*, *Breznakia*, *Bulleidia*, *Cantenisphaera*, *Dielma*, *Dubosiella*, *Erysipelothrix*, *Faecalibacillus*, *Faecalibaculum*, *Faecalicoccus*, *Faecalitalea*, *Floccifex*, *Holdemanella*, *Holdemania*, *Ileibacterium*, *Intestinibaculum*, *Longibaculum*, *Longicatena*, *Solobacterium* and *Stecheria* [13].

Erysipelotrichaceae, along with other Firmicutes such as *Lachnospiraceae* and *Ruminococcaceae*, dominate the anterior end of the hindgut of the marine herbivorous fish *Kyphosus sydneyanus* [14]. *K. sydneyanus* belongs to an ecologically important family of

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Keywords: Firmicutes; *Erysipelotrichaceae*; *Tannockella kyphosi*; hindgut fermenter; seaweed; herbivorous fish.
Abbreviations: ANI, average nucleotide identity; CDS, coding sequence; *is*DDH, *in silico* DNA–DNA hybridization; SCFA, short chain fatty acid; YCFA, yeast extract–caseine hydrolysate–volatile fatty acids; YCFA-M, modified YCFA medium containing mannitol.
The GenBank/EMBL/DDBJ accession number for the near-complete 16S rRNA gene sequence of *Tannockella kyphosi* strain BP52G is MG827409. The NCBI accession number for the whole-genome sequence of *Tannockella kyphosi* strain BP52G is CP088239.
Two supplementary figures and two supplementary tables are available with the online version of this article.

marine fishes (Kyphosidae) that feed on macroalgae and rely on their gut microbes for nutrition [15–17]. To our knowledge, no cultured bacterial species isolated from the gut of any kyphosid has been described in the literature, and the specific functions of *K. sydneyanus* gut microbiota are not well understood.

Here we describe the isolation and the physiological and genomic characteristics of bacterial strain BP52G^T isolated from the hindgut of the marine herbivorous fish *K. sydneyanus*. Through comparative 16S rRNA gene sequence analysis it was found that isolate BP52G^T is related to members of the family *Erysipelotrichaceae*. Phenotypic testing, phylogenetic comparative methods and chemotaxonomic analysis were conducted to further characterize BP52G^T. Based on these results, we propose that strain BP52G^T represents a novel species of a new genus in the family *Erysipelotrichaceae*.

ISOLATION AND ECOLOGY

Strain BP52G^T was isolated from the hindgut of the marine herbivorous fish *K. sydneyanus*. This fish lives in the temperate coastal waters of New Zealand and Australia. The stomach contents of these fishes show that they are strictly herbivorous and consume predominantly brown macroalgae such as *Ecklonia radiata* [18, 19]. Dietary seaweed is degraded in the fermentative hindgut, and the major short chain fatty acid (SCFA) products are acetate, propionate and butyrate [17]. For this study, fish were caught by spearfishing near Aiguilles Island at the northern end of Great Barrier Island in the Hauraki Gulf, New Zealand. Fish not killed outright by the spear were euthanized immediately by pithing following the Australian and New Zealand Council for the Care of Animals in Research and Training (https://anzccart.org.nz/) guidelines. Fish collection was conducted under University of Auckland Ethics approval AEC-001949. Fish were processed aboard the University of Auckland research vessel RV Hawere. The gut was removed and divided into five segments, numbered I–V, as described previously [20]. The contents of section IV of the hindgut of a freshly captured fish were transported to the laboratory under anaerobic conditions. The tube of gut contents was placed inside a vinyl anaerobic chamber (Coy Laboratory Products) filled with N_2:H_2:CO_2 (87.5:7.5:5) (H_2 levels at 3% inside the chamber). Solids were allowed to settle and then 100 µL of the supernatant fluid was plated on modified YCFA (yeast extract–casein hydrolysate–volatile fatty acids) [21] agar plates and incubated in an anaerobic jar under an atmosphere of 47.5% (v/v) N_2, 47.5% CO_2 and 5% H_2. One of the colonies isolated as a pure culture was designated isolate BP52G^T. Colonies appeared after 5 days of incubation at 19 °C. The culture was stored as a 25% glycerol suspension and separately as a stab in agar medium at −80 °C.

Our modified YCFA medium (YCFA-M) contained (per 100 ml): 1 g tryptone, 0.25 g yeast extract, 0.2 g mannitol, 0.4 g $NaHCO_3$, 0.05 g cysteine hydrochloride, 0.023 g K_2HPO_4, 0.023 g KH_2PO_4, 0.023 g $(NH_4)_2SO_4$, 0.045 g NaCl, 0.009 g $MgSO_4{\cdot}7H_20$, 5.7 mg $CaCl_2{\cdot}2H_2O$, 0.1 mg resazurin, 0.05 mg menadione, 0.01 g haemin, 1 µg biotin, 1 µg cobalamin, 3 µg *para*-amino-benzoic acid, 10 µg pyridoxamine, and 5 µg each of folic acid, pyridoxine, thiamine, riboflavin, nicotinic acid, lipoic acid and calcium pantothenate. SCFAs were added to the medium at concentrations of 30 mM acetate, 8 mM propionate, 4.3 mM butyrate, 0.9 mM each of valerate, isovalerate and DL-α-methyl butyric acid and 1.1 mM isobutyrate. In addition, a mixture of trace elements was added: $CuSO_4{\cdot}4H_2O$ (0.4 µg), MnSO4·4H_2O (5 µg), Ni(II)Cl_2 (0.02 µg), FeSO4 (1 µg), Co(II)$(NO_3)_2{\cdot}$6H2O (0.02 µg) and H_2MoO_4 (0.02 µg). Sterile $NaHCO_3$, cysteine, vitamins, haemin, SCFAs and mannitol were added after autoclaving.

16S rRNA PHYLOGENY

Colony PCR was performed and the 16S rRNA gene was amplified using the primer set of forward primer F_0008 (5′- AGAGTTT-GATYMTGGCTCAG-3′) and reverse primer R_1510 (5′-ACGGYTACCTTGTTACGACTT-3′), and sequenced in two directions using Sanger sequencing chemistry (ABI PRISM 3130XL Genetic Analyzer, Applied Biosystems). The full-length 16S rRNA gene was compared to the NCBI 16S rRNA gene database using the Basic Local Alignment Search Tool for identification (Table 1). Sequences of strain BP52G^T and related members of the family *Erysipelotrichaceae* were aligned using MUSCLE [22]. A maximum-likelihood phylogenetic tree was reconstructed based on the alignment using the PhyML method [23]. The appropriate substitution model was selected using jModelTest2 [24]. The maximum-likelihood tree suggests that strain BP52G^T is most closely related to members of the genus *Erysipelatoclostridium* (Fig. 1). A neighbour-joining tree based on the same alignment and a tree based on the concatenation of six housekeeping genes (*rpoB*, *recA*, *infB*, *gyrB*, *fusA*, *dnaK*) also support this relationship (Figs S1 and

Table 1. Percentage of 16S rRNA sequence similarity to strain BP52G^T

	Species name	Similarity (%)	Accession no.
1	*Tannockella kyphosi*	100	MG827409.1
2	*Clostridium saccharogumia*	93.88	NR_043550.1
3	*Coprobacillus catenaformis*	92.62	NR_024733.1
4	*Longibaculum muris*	91.18	NR_144615.1

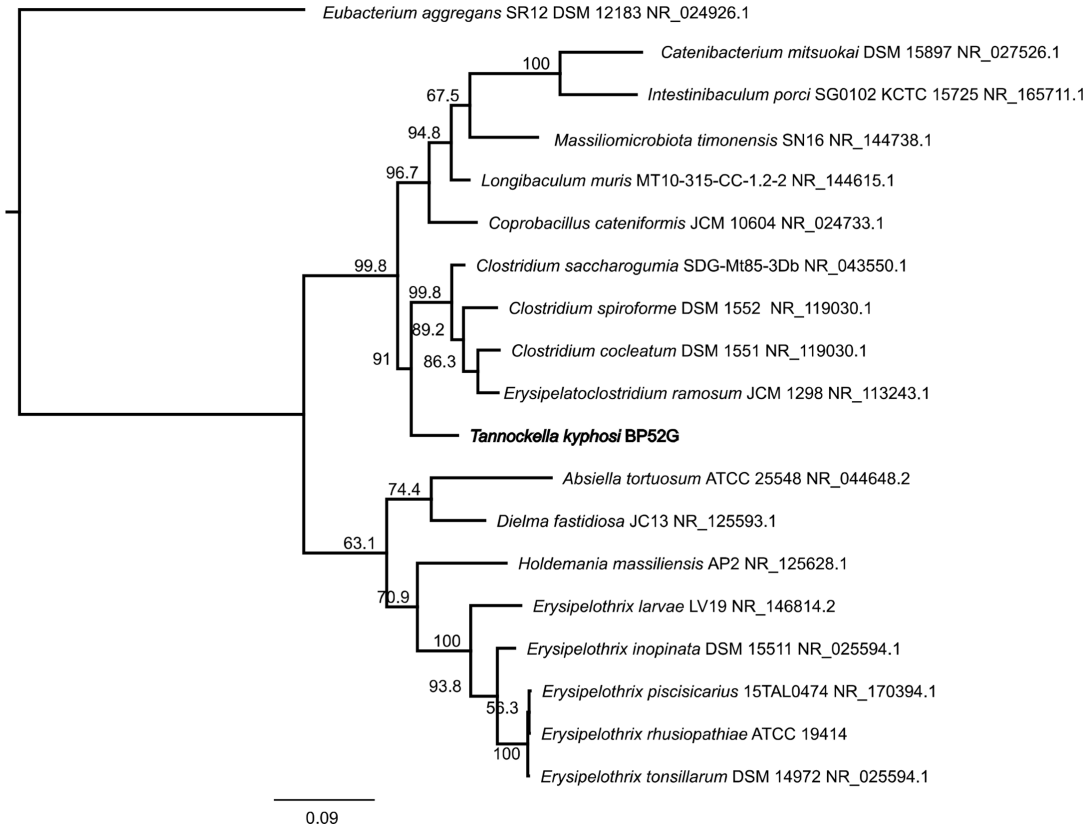

Fig. 1. Phylogenetic tree inferred by the maximum-likelihood method based on 16S rRNA gene sequences showing the relationship between strain BP52G^T and the most closely related organisms from the family *Erysipelotrichaceae*. The 16S rRNA gene sequence of *Eubacterium aggregans* SR12^T was used as an outgroup. This phylogenetic tree was generated using the PhyML method [23] based on the Jukes and Cantor model [37] using 1000 bootstraps, the percentages for which are shown next to the branches. Scale represents 0.09 nucleotide substitutions per site.

S2, available in the online version of this article) [25, 26]. The alignment and phylogenetic trees were produced using Geneious Prime 2020.1.2 (www.geneious.com).

Based on the 16S rRNA gene sequence comparisons, strain BP52G^T is 93.34% identical to the closest described species, *Clostridium saccharogumia* SDG-Mt85-3 Db^T (DSM 17460^T) (Table 1). This suggests that strain BP52G^T differs substantially from recognised genera of this family, which is not surprising given the environment from which it was isolated. Members of the gut microbiota of *K. sydneyanus* are likely under selection to use the abundant carbon substrates available in the gut of the host, which include carbohydrate polymers found in brown algal seaweed (i.e. laminarans, alginates, fucoidans containing mannuronic acids, guluronic acids, 2-O and 4-O sulfated residues with poor fermentability [27]) and mannitol. These marine seaweed polymers differ structurally from the fermentable polymers of terrestrial plants, and likewise mannitol is not an abundant carbohydrate in terrestrial plants.

Given this level of identity with 16S rRNA gene sequences of other genera of *Erysipelotrichaceae*, and considering the threshold for determining the affiliation of a bacterial strain to an existing genus is ~94.5% or higher [28], the classification of a novel genus and species in the family *Erysipelotrichaceae* is warranted to accommodate strain BP52G^T.

GENOME FEATURES

High molecular weight genomic DNA was extracted from strain BP52G^T using the PacBio DNA extraction protocol (www.pacb.com/wp-content/uploads/2015/09/SharedProtocol-Extracting-DNA-usinig-Phenol-Chloroform.pdf) [29]. All steps were performed inside an anaerobic chamber until the lysis step to avoid potential degradation of DNA by oxygen-activated DNases [30]. The genome of strain BP52G^T was sequenced using PacBio RSII (Pacific Biosciences) by Macrogen (Seoul, Republic of Korea). The sequencing coverage was 412×. The resulting reads were *de novo* assembled by Nextgen Bioinformatic Services (Palmerston, New Zealand) using Canu version 1.7 [31]. The genome was annotated using the Joint Genome Institute ntegrated Microbial

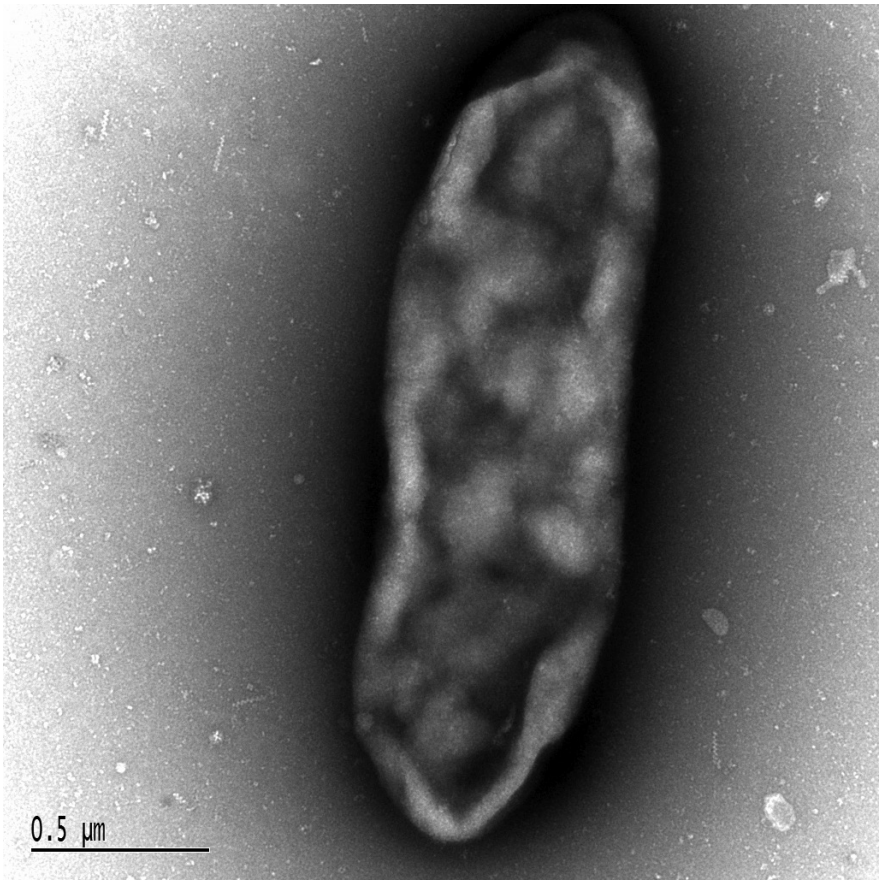

Fig. 2. Scanning electron microscope image of strain BP52G^T. Cells were imaged after culturing in anaerobic conditions for 24 h at 24 °C in YCFA-M medium.

Genomes and Microbiomes annotation pipeline version 5.0.0 [32]. The genome sequence was submitted to the GenBank (www.ncbi.nlm.nih.gov) database.

The assembly resulted in a single circular chromosome with a length of 2260890 bp and 2306 features were annotated: 2129 were protein-coding sequences, 15 were rRNA genes, and 48 were tRNA genes. The remaining features included two CRISPRs and two ncRNA genes, among other miscellaneous features. The G+C content of strain BP52G^T was 30.7 mol%. The average nucleotide identity (ANI) values were calculated by the OrthoANI using EZbiocloud www.ezbiocloud.net/tools/ani) [33]. *In silico* DNA–DNA hybridization (*is*DDH) was estimated using the Genome-to-Genome Distance Calculator [34]. When the whole genome sequence of strain BP52G^T is compared to closely related strains, both the ANI values and the *is*DDH values were low (Tables S1 and S2). The closest relative to BP52G^T in the analyses was *Erysipelatoclostridium ramosum* JCM 1298^T with ANI and *is*DDH values of 72.65 and 20.9%, respectively. These results indicate that strain BP52G^T represents a novel species and likely a new genus.

PHYSIOLOGY AND CHEMOTAXONOMY

The strain was cultivated in YCFA-M medium under anaerobic growth conditions at 20 °C and pH 7.8. Bacterial colony characteristics were determined after streaking the bacteria on YCFA-M agar plates followed by 5 days of incubation. Gram staining was performed using a kit (Difco) according to the manufacturer's protocol. During the exponential growth of the bacterium, cell morphology was examined by scanning electron microscopy. BP52G^T was incubated separately under aerobic and anaerobic conditions to determine aerotolerance. Further, the strain was grown at 12, 16, 20, 24, 28 and 37 °C to determine the range of growth under anaerobic conditions. The YCFA-M medium was adjusted to pH range of pH 6.9–8.5 to determine the growth of the strain at different pH levels. Salinity (NaCl) tolerance was determined using YCFA-M medium containing various concentrations of NaCl.

A Gram stain of a log phase broth culture showed that the cells were weakly Gram-positive and appeared to be rods occasionally in chains. When cells were viewed after negative staining with uranyl acetate, they appeared as rods approximately

Table 2. Biochemical features that differentiate strain BP52G^T from its closest phylogenetic neighbours

Strains: 1, BP52G^T (data from this study); 2, *Clostridium saccharogumia* [38]; 3, *Coprobacillus catenaformis* [39]; 4, *Longibaculum muris* [40]. +, Positive; –, negative; W, weak; ND, no data available.

Characteristic	1	2	3	4
Isolation source	Fish hindgut	Human faeces	Human faeces	Caeca of mouse
Cell diameter and length (μm)	0.7×2.0–3.0	0.9×2.0–3.0	0.4×1.2–1.7	>20
Oxygen requirement	Anaerobic	Anaerobic	Anaerobic	Anaerobic
Gram stain	+	+	+	ND
Motility	–	–	–	ND
Endospore formation	–	–	–	ND
Catalase	–	–	ND	ND
Oxidase	–	ND	ND	ND
Conditions for growth:				
Temperature (°C)	20–28	25–45	37	ND
pH	6.9–8.5	6.0–9.0	7.6–7.7	7.0
Gelatin hydrolysis	–	ND	–	ND
Aesculin hydrolysis	–	ND	V (1/3)	ND
Acid production (API 20A) from:				
D-Glucose	W	+	+	–
D-Mannitol	W	–	–	–
Lactose	W	+	+	–
Sucrose	–	+	+	–
Maltose	–	–	+	–
Salicin	W	+	+	–
D-Sylose	–	ND	–	–
L-Arabinose	–	ND	–	–
Glycerol	–	ND	ND	–
Cellobiose	W	+	+	–
D-Mannose	W	+	+	–
Melezitose	–	ND	–	–
Raffinose	–	–	–	–
D-Sorbitol	–	ND	–	–
L-Rhamnose	–	–	–	–
Trehalose	–	–	+	–
Enzyme activity (API 32A):				
Urease	–	ND	ND	–
Arginine dihydrolase	–	ND	ND	–
α-Galactosidase	+	–	ND	+
β-Galactosidase	–	ND	ND	–
6-Phospho-β-galactosidase	+	ND	ND	–
α-Glucosidase	–	ND	ND	–

Continued

Table 2. Continued

Characteristic	1	2	3	4
β-Glucosidase	–	–	ND	–
α-Arabinosidase	–	ND	ND	–
β-Glucuronidase	–	ND	ND	+
N-Acetyl-β-glucosaminidase	–	ND	ND	–
Glutamic acid decarboxylase	–	ND	ND	–
α-Fucosidase	–	ND	ND	–
Alkaline phosphatase	–	ND	ND	–
Arginine arylamidase	–	ND	ND	–
Proline arylamidase	–	ND	ND	–
Leucyl glycine arylamidase	–	ND	ND	–
Phenylalanine arylamidase	–	ND	ND	–
Leucine arylamidase	–	ND	ND	–
Pyroglutamic acid arylamidase	–	ND	ND	+
Tyrosine arylamidase	–	ND	ND	–
Alanine arylamidase	–	ND	ND	–
Glycine arylamidase	–	ND	ND	–
Histidine arylamidase	–	ND	ND	–
Glutamyl glutamic acid arylamidase	–	ND	ND	–
Serine arylamidase	–	ND	ND	–
G+C content (mol%)	30.7	30.7±0.8	32.0–34.0	30.8

0.7 µm wide and up to 6 µm long, sometimes with one or both ends slightly pointed (Fig. 2). No endospores or flagella were seen. Colony morphology on YCFA-M agar appeared white, opaque, and shiny with a diameter about 1 mm after a week of anaerobic growth at 19 °C. The strain grew in the temperature range 20–28 °C (optimum, 24 °C) at a pH range of pH 6.9–8.5 (optimum, pH 7.8) and tolerated up to 1% (w/v) NaCl. There was no growth at 12, 16 and 37 °C. This is not unexpected, as the ambient temperature range at the location where the isolate where the host of the isolate was obtained is 17–23 °C [17].

Table 3. Fermentation products of strain BP52G^T when grown in YCFA-M medium

Fermentation product	Initial conc. (mM)	Final conc. (mM)	Change in conc. (mM)
Formate	1.76	9.20	7.44*
Acetate	24.54	27.18	2.64*
Propionate	7.54	7.56	0.02
Butyrate	4.29	4.29	0
Isobutyrate	1.05	1.05	0
Valerate	1.08	1.08	0
Isovalerate	1.00	1.05	0.05
Caproate	0	0	0
Enanthoate	0	0	0
Succinate	0	0	0
Lactate	0	0	0

*Value significantly (p<0.001) different from initial concentration.

Table 4. Cellular fatty acid contents (%) of strain BP52G^T and its closest relatives

Values are percentages of the total fatty acids detected. –, Not detected. The predominant fatty acids are highlighted using bold type.

Fatty acid	BP52G^T	*Clostridium saccharogumia*	*Coprobacillus catenaformis*	*Longibaculum muris*
$C_{12:0}$	2.5	–	–	–
$C_{14:0}$	**7.7**	3.6	–	–
$C_{15:0}$	2.1	–	–	–
$C_{16:0}$	**33.2**	**20.2**	–	**30.1**
$C_{17:0}$	1.6	–	–	–
$C_{18:0}$	**8.4**	**12.6***	–	**6.6**
$C_{18:1n-7t}$	4.4	–	–	–
$C_{15:1n-5c}$	1.2	–	–	–
$C_{16:1n-7c}$	2.0	1.7	–	**8.9**
$C_{16:1n-7t}$	**13.5**	–	–	–
$C_{17:1n-7c}$	2.3	–	–	–
$C_{18:1n-7c}$	1.0	**12.9***	–	**9.9**
$C_{18:1n-9c}$	**15.3**	**9.3**	–	**15.4**
$C_{18:1n-7t}$	4.4	–	–	–

*Part of summed percentages that represent groups of two of three fatty acids not separable under the experimental conditions used.

The optimum pH for growth identified in this study is within the range of pH value found in section IV of the hindgut of *K. sydneyanus* [35]. The strain grew well in YCFA-M broth as well as agar only under anaerobic conditions, confirming that the strain was an obligate anaerobe. Like other obligate anaerobes, isolate BP52G^T was negative for catalase and oxidase activity (Table 2).

After growing the strain BP52G^T in YCFA-M broth, cells were harvested at the end of log phase for cellular fatty acid analysis. Fatty acids were extracted, purified and methylated according to Lepage and Roy [36] and identified and analysed using GC (Agilent 7890A) according to the manufacturer's instructions. Fermentation end-products after growth in YCFA-M medium were identified using a Shimadzu gas chromatography system (Nexus) equipped with a flame ionization detector and fitted with an HP-1 column (30 m×0.25 mm ID ×0.25 µm film thickness; Agilent Technologies). The fermentation products produced during growth in YCFA-M medium were formate and acetate (Table 3). The major cellular fatty acids of strain BP52G^T (at >5% of the total) were $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{16:1n-7t}$ and $C_{18:1n-9c}$ (Table 4).

Enzymatic activities and the utilization of various carbon sources were determined using API20A and API32A kits (bioMerieux) according to the manufacturer's instructions. Positive enzymatic activities for α-galactosidase and 6-phospho-β-galactosidase were observed for BP52G^T, differentiating it from *Clostridium saccharogumia* SDG-Mt85-3 Db^T and *Longibaculum muris* MT10-315-CC-1.2-2^T (Table 2). Fermentation of carbohydrates were mostly negative (using test kit API 20A), similar to *Longibaculum muris* MT10-315-CC-1.2-2^T, but a weak fermentation reaction was produced with D-glucose, D-mannitol, lactose, salicin, cellobiose and D-mannose. All of these sugars except mannitol were also used by its closest relatives *Clostridium saccharogumia* SDG-Mt85-3 Db^T and *Coprobacillus catenaformis* JCM10604^T.

DESCRIPTION OF *TANNOCKELLA* GEN. NOV.

Tannockella (Tan.nock.el'la. N.L. fem. dim. n. *Tannockella* named for the New Zealand microbiologist Gerald Tannock for his pioneering, numerous and important contributions in the field of human gut microbiome research).

Cells of the only described species are weakly Gram-positive (easily decolourized and may appear Gram-negative). The cells are rods (2.0–3.0 µm long and 0.7 µm wide) sometimes with one or two pointed ends. Colonies on YCFA-M agar appear white, opaque and shiny with a diameter about 1 mm after a week growing anaerobically at 19 °C. No endospores or flagella were seen. The type species is *Tannockella kyphosi*.

DESCRIPTION OF *TANNOCKELLA KYPHOSI* SP. NOV.

Tannockella kyphosi (ky.pho'si. N.L. gen. n. *kyphosi*, of the fish genus *Kyphosus*).

Cells are rods approximately 3 µm long and 0.7 µm wide growing mainly as single cells. The optimum NaCl concentration required for growth is 0.045% (w/v). Negative for catalase, oxidase, aesculin, indole production, nitrate reduction, urease reaction and gelatin hydrolysis. There is a positive reaction for α-galactosidase and 6-phospho-β-galactosidase, but not for β-galactosidase, arginine arylamidase, histidine arylamidase, α-glucosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, glutamic acid decarboxylase, α-fucosidase, alkaline phosphatase, proline, leucyl glycine, phenylalanine, leucine, pyroglutamic acid, tyrosine, alanine, glycine, glutamyl glutamic acid or serine. The cells grow well on YCFA-M medium. Growth occurs in the temperature range 20–28 °C (optimum, 24 °C), at a pH range of pH 6.9–8.5 (optimum, pH 7.8). The fermentation products produced during growth in YCFA-M medium are formate and acetate. Cells grow only under reducing conditions in the absence of oxygen. The major cellular fatty acids present (at >5% of the total) are $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{16:1n-7t}$ and $C_{18:1n-9c}$.

The type strain is strain BP52G^T (=NZRM 4757^T=JCM 34692^T), isolated at the School of Biological Sciences, University of Auckland, New Zealand. Strains were isolated from the fermentative hindgut contents of the herbivorous marine fish *Kyphosus sydneyanus*.

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Author contributions
B.P. and A.M.R., planned and designed the study and performed the experiments. E.M.W., performed the bioinformatics. W.L.W., K.D.C., B.P. and A.M.R., performed the field work and sampling. B.P., A.M.R. and E.M.W., wrote the manuscript and all authors contributed to final preparation.

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

Ethical statement
Fish collection was conducted under University of Auckland Animal Ethics approval AEC-001949.

References

1. Verbarg S, Rheims H, Emus S, Frühling A, Kroppenstedt RM, *et al*. *Erysipelothrix inopinata* sp. nov., isolated in the course of sterile filtration of vegetable peptone broth, and description of *Erysipelotrichaceae* fam. nov. *Int J Syst Evol Microbiol* 2004;54:221–225.
2. Sneath PHA, McGowan V, Skerman VBD. Approved lists of bacterial names. *Int J Syst Evol Microbiol* 1980;30:225–420.
3. Migula W. *System der Bakterien*. Jena: Verlag von Gustav Fischer; 1900.
4. Alcaide M, Messina E, Richter M, Bargiela R, Peplies J, *et al*. Gene sets for utilization of primary and secondary nutrition supplies in the distal gut of endangered Iberian lynx. *PLoS One* 2012;7:e51521.
5. Han I, Congeevaram S, Ki DW, Oh BT, Park J. Bacterial community analysis of swine manure treated with autothermal thermophilic aerobic digestion. *Appl Microbiol Biotechnol* 2011;89:835–842.
6. Egert M, Wagner B, Lemke T, Brune A, Friedrich MW. Microbial community structure in midgut and hindgut of the humus-feeding larva of *Pachnoda ephippiata* (*Coleoptera: Scarabaeidae*). *Appl Environ Microbiol* 2003;69:6659–6668.
7. Tegtmeier D, Riese C, Geissinger O, Radek R, Brune A. *Breznakia blatticola* gen. nov. sp. nov. and *Breznakia pachnodae* sp. nov., two fermenting bacteria isolated from insect guts, and emended description of the family *Erysipelotrichaceae*. *Syst Appl Microbiol* 2016;39:319–329.
8. Cox LM, Yamanishi S, Sohn J, Alekseyenko AV, Leung JM, *et al*. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell* 2014;158:705–721.
9. Kaakoush NO. Insights into the role of *Erysipelotrichaceae* in the human host. *Front Cell Infect Microbiol* 2015;5:84.
10. Gao Y-M, Zou K-S, Zhou L, Huang X-D, Li Y-Y, *et al*. Deep insights into gut microbiota in four carnivorous coral reef fishes from the South China Sea. *Microorganisms* 2020;8:E426.
11. Rimoldi S, Terova G, Ascione C, Giannico R, Brambilla F. Next generation sequencing for gut microbiome characterization in rainbow trout (*Oncorhynchus mykiss*) fed animal by-product meals as an alternative to fishmeal protein sources. *PLoS One* 2018;13:e0193652.
12. Sylvain F-É, Derome N. Vertically and horizontally transmitted microbial symbionts shape the gut microbiota ontogenesis of a skin-mucus feeding discus fish progeny. *Sci Rep* 2017;7:5263.
13. Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M. List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *Int J Syst Evol Microbiol* 2020;70:5607–5612.
14. Pardesi B, Roberton AM, Lee KC, Angert ER, Rosendale DI, *et al*. Distinct microbiota composition and fermentation products indicate functional compartmentalization in the hindgut of a marine herbivorous fish. *Mol Ecol* 2022;31:2494–2509.

15. Clements KD, Angert ER, Montgomery WL, Choat JH. Intestinal microbiota in fishes: what's known and what's not. *Mol Ecol* 2014;23:1891–1898.
16. Clements KD, Gleeson VP, Slaytor M. Short-chain fatty acid metabolism in temperate marine herbivorous fish. *J Comp Physiol B* 1994;164:372–377.
17. Mountfort DO, Campbell J, Clements KD. Hindgut fermentation in three species of marine herbivorous fish. *Appl Environ Microbiol* 2002;68:1374–1380.
18. Moran D, Clements KD. Diet and endogenous carbohydrases in the temperate marine herbivorous fish Kyphosus sydneyanus. *J Fish Biol* 2002;60:1190–1203.
19. Clements KD, Choat JH. Comparison of herbivory in the closely-related marine fish genera *Girella* and *Kyphosus*. *Marine Biology* 1997;127:579–586.
20. Johnson KS, Clements KD. Histology and ultrastructure of the gastrointestinal tract in four temperate marine herbivorous fishes. *J Morphol* 2022;283:16–34.
21. Duncan SH, Hold GL, Harmsen HJM, Stewart CS, Flint HJ. Growth requirements and fermentation products of *Fusobacterium prausnitzii*, and a proposal to reclassify it as *Faecalibacterium prausnitzii* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 2002;52:2141–2146.
22. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004;32:1792–1797.
23. Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, *et al*. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 2010;59:307–321.
24. Darriba D, Taboada GL, Doallo R, Posada D. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 2012;9:772.
25. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 1993;10:512–526.
26. Le SQ, Gascuel O. An improved general amino acid replacement matrix. *Mol Biol Evol* 2008;25:1307–1320.
27. Parkar S, Blatchford P, Kim C, Rosendale D, Ansell J. New and tailored prebiotics: established applications. In: *Probiotics and Prebiotics: Current Research and Future Trends*. Caister Academic Press, 2015. pp. 289–314.
28. Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, *et al*. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nat Rev Microbiol* 2014;12:635–645.
29. Sambrook J, Russell DW. Purification of nucleic acids by extraction with phenol:chloroform. *CSH Protoc* 2006;2006:pdb.prot4455.
30. Martin JH, Savage DC. Degradation of DNA in cells and extracts of the obligately anaerobic bacterium *Roseburia cecicola* upon exposure to air. *Appl Environ Microbiol* 1988;54:1619–1621.
31. Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, *et al*. Canu: scalable and accurate long-read assembly via adaptive *k*-mer weighting and repeat separation. *Genome Res* 2017;27:722–736.
32. Chen I-MA, Chu K, Palaniappan K, Pillay M, Ratner A, *et al*. IMG/M v.5.0: an integrated data management and comparative analysis system for microbial genomes and microbiomes. *Nucleic Acids Res* 2019;47:D666–D677.
33. Yoon SH, Ha SM, Lim J, Kwon S, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281–1286.
34. Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. *Nucleic Acids Res* 2022;50:D801–D807.
35. Zemke-White WL, Clements KD, Harris PJ. Acid lysis of macroalgae by marine herbivorous fishes: myth or digestive mechanism? *J Exp Mar Biol Ecol* 1999;233:95–113.
36. Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. *J Lipid Res* 1986;27:114–120.
37. Jukes TH, Cantor CR. Evolution of protein molecules. In: Munro HN (eds). *Mammalian Protein Metabolism*. Academic Press; 1969. pp. 21–132.
38. Clavel T, Lippman R, Gavini F, Doré J, Blaut M. *Clostridium saccharogumia* sp. nov. and Lactonifactor longoviformis gen. nov., sp. nov., two novel human faecal bacteria involved in the conversion of the dietary phytoestrogen secoisolariciresinol diglucoside. *Syst Appl Microbiol* 2007;30:16–26.
39. Kageyama A, Benno Y. *Coprobacillus catenaformis* gen. nov., sp. nov., a new genus and species isolated from human feces. *Microbiol Immunol* 2000;44:23–28.
40. Lagkouvardos I, Pukall R, Abt B, Foesel BU, Meier-Kolthoff JP, *et al*. The Mouse Intestinal Bacterial Collection (miBC) provides host-specific insight into cultured diversity and functional potential of the gut microbiota. *Nat Microbiol* 2016;1:16131.