Peptoniphilus catoniae sp. nov., isolated from a human faecal sample from a traditional Peruvian coastal community

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A novel Gram-stain-positive, coccus-shaped, obligately anaerobic bacterium was isolated from a faecal sample obtained from an individual in a traditional community located off the southern coast of Peru. Comparative 16S rRNA gene sequence analysis showed the novel bacterium belonged to the genus *Peptoniphilus* but showed no particular relationship with any species, demonstrating less than 91 % 16S rRNA gene sequence similarity with all members of the genus. The major cellular fatty acids of the novel isolate were determined to be $C_{10:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:1}\omega9c$ and $C_{18:2}\omega6,9c$/anteiso-$C_{18:0}$. The DNA G+C content was 34.4 mol%. End-products of metabolism from peptone-yeast-glucose broth (PYG) were determined to be acetate and butyrate. Based on the phenotypic, chemotaxonomic and phylogenetic results, the organism represents a novel species of the genus *Peptoniphilus*, for which the name *Peptoniphilus catoniae* sp. nov. is proposed. The type strain is M6.X2D^T (=DSM 29874^T=CCUG 66798^T).

Molecular inventories based mainly on the use of 16S rRNA gene sequencing have provided tremendous insights into the microbial diversity and the richness of taxa present in the human gastrointestinal tract (Eckburg *et al.*, 2005; Turnbaugh *et al.*, 2007). However, the majority of these studies have focused on western populations implementing the use of culture-independent methods (Gordon, 2012; Zhou *et al.*, 2014), but in order to truly evaluate the core microbiome, individuals from a variety of geographical regions with diverse diets must also be included in these investigations. Studies are now revealing that the microbiome of indigenous communities may be significantly different from those derived from westernized and urbanized communities (Blaser *et al.*, 2013; Contreras *et al.*, 2010). In our continuing studies of traditional communities in Peru (Obregon-Tito *et al.*, 2015; Patel *et al.*, 2015; O'Neal *et al.*, 2015), a novel, *Peptoniphilus*-like organism was isolated.

Johnson and colleagues recently created the family *Peptoniphilaceae* that includes a group of organisms commonly referred to as the Gram-Positive Anaerobic Cocci (Murdoch, 1998) belonging to the genera *Anaerococcus*, *Anaerosphaera*, *Finegoldia*, *Gallicola*, *Helcococcus*, *Murdochiella*, *Parvimonas* and *Peptoniphilus* (Johnson *et al.*, 2014). At the time of writing, the genus *Peptoniphilus* contains 13 species with validly published names that have been isolated from human or animal sources (Ezaki & Kawamura, 2009; Parte, 2014). Based on the phenotypic, chemotaxonomic, and phylogenetic results from the characterisation of the *Peptoniphilus*-like organism from a traditional community in Peru, a novel species of the genus *Peptoniphilus* is proposed.

Strain M6.X2D^T was isolated from a freshly voided faecal sample obtained from one member of a traditional coastal Peruvian community as part of a study to examine microbial diversity of geographically remote, traditional native communities from Peru. The sample was collected, processed anaerobically, and transported on ice back to the Lawson Systematics laboratory for further processing.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of strain M6.X2D^T is KR911910.

An enrichment was prepared anaerobically using 1.0 ml faecal slurry in a sealed serum bottle containing 100 ml Medium 2 (Hobson, 1969) supplemented with xylan. The medium comprised (per 100 ml distilled water): Casitone (1.0 g), yeast extract (0.25 g), minerals solution (A) (15 ml), minerals solution (B) (15 ml), clarified rumen fluid (20 ml), resazurin (0.0001 g), sodium lactate (70 %, w/v) (1.0 g), xylan (0.2 g), cysteine HCl (0.05 g), sodium bicarbonate (0.4 g) and distilled water to 100 ml. Minerals solution (A) contained (per 1000 ml): K_2HP0_4 (3.0 g). Minerals solution (B) contained (per 1000 ml): KH_2P0_4 (3.0 g), $(NH_4)_2S0_4$ (6.0 g), NaCl (6.0 g), $MgS0_4 . 7H_20$ (0.6 g) and $CaCl_2$ (0.6 g). The enrichment was incubated at 37 °C for 14 days with a gas mix of 5 % hydrogen, 10 % carbon dioxide and 85 % nitrogen to maintain anoxic conditions. Throughout the 14 day period, a sample of the enrichment was transferred onto Medium 2 agar and after a 5 day incubation, colonies were picked and sub-cultured onto the same medium. All isolates were then sub-cultured on agar plates of BD Bacto Brain Heart Infusion (BHI) supplemented with 5 % defibrinated sheep blood (pH 7.4) until pure cultures were obtained for all isolates. One of the resulting isolates was designated M6.X2D^T. After primary isolation, this strain was continually sub-cultured on BHI plus 5 % sheep blood medium at 37 °C. Other classical phenotypic tests were performed as described by Tindall *et al.* (2007). Testing for the presence of spores was carried out using both the ethanol and heat shock methods as described previously (Koransky *et al.*, 1978), following growth in BHI.

Unless indicated otherwise, morphological, physiological, molecular and chemotaxonomic tests were performed with cells grown on anoxic modified peptone-yeast extract (PYG) broth medium. For phylogenetic analysis, DNA of strain M6.X2D^T was extracted using the UltraClean Microbial DNA Isolation kit (MoBio Laboratories) according to the manufacturer's instructions. The 16S rRNA gene was amplified via PCR using universal primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′), and the resulting amplicon was purified using Exo-SapIt (USB Corporation). The 16S rRNA gene sequence was concatenated using Sequencher (version 5.1 sequence analysis software; Gene Codes Corporation, Ann Arbor, MI, USA) and manually read for correct alignment and to identify sequencing errors. The nearest known relatives of strain M6.X2D^T were determined using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012) based upon pairwise sequence similarity calculations using an alignment algorithm (Myers & Miller, 1988). The sequences were retrieved and aligned with strain M6.X2D^T using CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic analysis was performed using the bootstrap algorithm based on 1000 replicates in the MEGA software package (version 5) (Tamura *et al.*, 2011) using the neighbour-joining method (Saitou & Nei, 1987). Strain M6.X2D^T is phylogenetically a member of the phylum *Firmicutes*, specifically placed within the genus *Peptoniphilus*. The highest pairwise 16S rRNA gene sequence similarities with strain M6.X2D^T were obtained with *Peptoniphilus koenoeneniae* WAL 18898^T (91.0 %) and *Peptoniphilus asaccharolyticus* CCUG 9988^T (90.6 %), with all other values being below 90 %. However, the topology of the phylogenetic tree demonstrates that the novel organism does not exhibit a particularly close relationship with any species of this genus. (Fig. 1).

The cell morphology of strain M6.X2D^T was observed using a phase-contrast microscope (CX41; Olympus) at ×1000 magnification after cells were grown for 6 days at 37 °C on BHI supplemented with 5 % sheep blood.

Using five experimental replicates, the DNA G+C content was determined according to Mesbah *et al.* (2011) via HPLC using an Alltech Prevail C18 reversed-phase column (150 × 4.6 mm, 5 μm particle size) with a mobile phase of 25 mM KH_2PO_4, pH 2.5/CH_3CN (96 : 4, v/v) at a flow rate of 1.0 ml min^{-1}, and a UV detector at 254 nm. Unmethylated λ phage DNA (cat. no. D1521; Promega) was used to calibrate the calculation, while single- stranded DNA from salmon testes (cat. no. D7656; Sigma) was used as a control. The DNA G+C content of strain M6.X2D^T was calculated to be 34.4 mol%.

Metabolic end-products were determined from cultures grown in anaerobic peptone-yeast-glucose broth (PYG). Sample analyses were carried out in duplicate on an Aminex HPX-87H organic acid analysis column (Bio-Rad), using ion-exclusion HPLC with 0.015 M HCl running buffer at a flow rate of 0.9 ml min^{-1}. Retention times and peak areas of fermentation products were compared to standards of acetate, butyrate, lactate, succinate, formate and propionate. Metabolic end-products measured in amounts greater than 2 mM were defined as major products. End-products for strain M6.X2D^T were determined to be acetate and butyrate. The results are consistent with the description of the genus *Peptoniphilus*.

For biochemical characterization, API Rapid ID 32A test systems (bioMérieux) using 5-day-old cultures were used following the manufacturer's instructions except that the strips were incubated anaerobically instead of aerobically and cupules were overlaid with mineral oil to maintain anoxic conditions. Additional biochemical characterizations were carried out using the AN MicroPlate system (Biolog) following the manufacturer's instructions. Physiological characteristics were determined at varying temperatures (4.0–60 °C, in increments of ~5 °C) and pH (5.0–9.5, in increments of 0.5 pH units). To adjust the pH, the following buffers were prepared at a final concentration of 10 mM: sodium acetate (for pH 5.0), potassium phosphate (pH 6.0–8.0), Tris/HCl (pH 7.0–9.0) and sodium borate (pH 9.5). Salt tolerance was examined using different concentrations of NaCl [0.0–0.5 % w/v), and 1.0–9.0 % (w/v), in increments of 1.0 %]. Optimal growth conditions were determined by monitoring the optical density at 600 nm using a spectrophotometer (Spectronic 20D). An increase

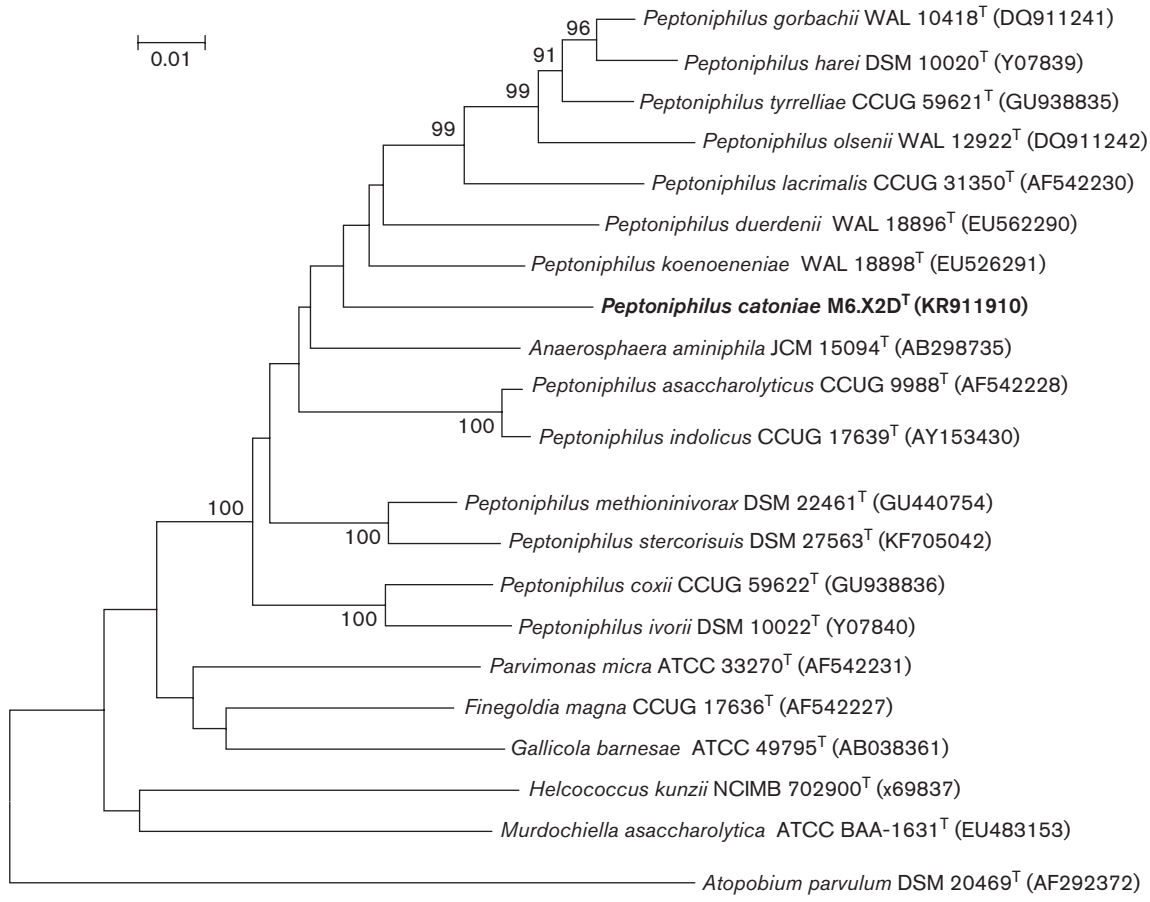


Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences indicating the position of strain M6.X2D^T with members of the family *Peptoniphilaceae* within the phylum *Firmicutes*. The tree was reconstructed using the neighbour-joining algorithm from MEGA (version 4) with *Atopobium parvulum* as the outgroup. Bootstrap values (%) were obtained with 1000 replicates and are displayed on their relative branches.

in OD_{600} of >0.1 was considered to indicate growth. All tests were performed in duplicate.

Biomass for fatty acid analysis was recovered from an agar plate of BHI amended with 5 % sheep blood after 72 h at 37 °C. Analysis was performed at the Center for Microbial Identification and Taxonomy (University of Oklahoma, Norman, Oklahoma). Fatty acid methyl esters were extracted using the Sherlock Microbial Identification System (MIDI) version 6.1 as described previously (Kämpfer & Kroppenstedt, 1996; Sasser, 1990). Analysis was performed using an Agilent Technologies 6890N gas chromatograph equipped with a phenyl methyl silicone-fused silica capillary column (HP-2 25 m × 0.2 mm × 0.33 µm film thickness) and a flame-ionization detector with hydrogen used as the carrier gas. The temperature program was initiated at 170 °C and increased at 5 °C min^{-1} reaching a final temperature of 270 °C. Fatty acids were identified and expressed in the form of percentages using the QBA1 peak naming database. The predominant fatty acids of strain M6.X2D^T were $C_{16:0}$ (19.1 %), $C_{18:1}\omega9c$ (18.3 %), $C_{14:0}$ (12.8 %), $C_{10:0}$ (12.1 %) and $C_{18:2}\omega6,9c$/anteiso-$C_{18:0}$ (10.8 %). The minor fatty acids were $C_{18:0}$/$C_{17:0}$ CYCLO (7.1 %), $C_{12:0}$ (6.2 %), $C_{18:1}\omega7c$ (3.2 %), $C_{15:0}$ (2.1 %), $C_{16:1}\omega7c$/$C_{16:1}\omega6c$ (1.9 %), $C_{17:1}\omega8c$ (1.6 %), $C_{19:0}$ CYCLO $\omega8c$/$C_{20:0}$ (0.8 %). Table 1 shows the fatty acid composition of strain M6.X2D^T and the type strains of some species of the genus *Peptoniphilus*. The results are consistent with observations reported in other studies regarding volatile short-chain fatty acid metabolic end-products generated by members of the genus *Peptoniphilus* (Ezaki & Kawamura, 2009; Johnson *et al.*, 2014; Ulger-Toprak *et al.*, 2012). The major products of $C_{16:0}$, $C_{18:1}\omega9c$, and $C_{18:2}\omega6,9c$/anteiso-$C_{18:0}$ in strain M6.X2D^T are consistent with most species of the genus *Peptoniphilus*, while the presence of $C_{10:0}$ distinguishes the isolate from other members of the genus.

Strain M6.X2D^T was recovered from human faecal material and is Gram-stain-positive, strictly anaerobic and coccus-shaped. When grown on blood agar, colonies are needle-point in size, beige, and circular with a smooth surface and raised elevation, and are non-haemolytic. Although

Table 1. Comparison of fatty acid profile of strain M6.X2D^T with other members of the genus *Peptoniphilus*

Strains: 1, M6.X2D^T (data from this study); 2, *P. stercorisuis* DSM 27563^T (Johnson *et al.*, 2014); 3, *P. asaccharolyticus* CCUG 9988^T; 4, *P. duerdenii* WAL 18896^T (Ulger-Toprak *et al.*, 2012); 5, *P. koenoeneniae* WAL 18898^T (Ulger-Toprak *et al.*, 2012); 6, *P. gorbachii* CCUG 53341^T; 7, *P. harei* CCUG 38491^T; 8, *P. indolicus* CCUG 17639^T; 9, *P. ivorii* 38492^T; 10, *P. lacrimalis* CCUG 31350^T; 11, *P. methioninivorax* NRRL B-23883^T (Rooney *et al.*, 2011); 12, *P. olsenii* CCUG 53342^T. Unless stated data was taken from www.CCUG.se. Predominant fatty acids are shown in bold, values <1 % are not shown. Profiles for *P. coxii* and *P. tyrrelliae* have not been performed.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11*	12
$C_{10:0}$	**12.1**			2.8			1.1					
$C_{12:0}$	6.2	2.2	2.3				1.3	1.5			1.2	2.0
iso-$C_{13:0}$ 2-OH								2.5				3.4
$C_{14:0}$	**12.8**	**22.4**	5.4	4.4	3.1	2.9	3.6	**12.5**	4.4	1.5	**10.9**	8.2
iso-$C_{15:0}$			2.6									
anteiso-$C_{15:0}$		1.7										
$C_{15:1}\omega 8c$		8.7						1.0				
$C_{15:0}$	2.1											
$C_{16:0}$	**19.1**	**15.6**	**14.4**	**33.0**	**22.6**	**24.0**	**18.8**	**11.3**	**29.5**	**20.2**	**25.1**	**13.1**
$C_{16:0}$ alde		**10.1**					1.8					0.7
$C_{16:1}\omega 5c$		2.9						1.5				1.1
$C_{16:1}\omega 7c$	1.9	**11.3**	3.9			2.7	3.3	5.3	1.0	3.4	**14.1**	4.8
$C_{16:1}\omega 7c$ DMA												1.5
$C_{16:1}\omega 9c$					3.3			3.1			3.4	1.3
$C_{17:1}\omega 9c$		1.1					3.0					
$C_{17:1}\omega 8c$	1.6											
$C_{17:0}$ iso-$C_{17:1}\omega 5c$			3.0			2.6		**18.5**				
$C_{17:0}$ DMA		3.0										
iso-$C_{17:1}$/$C_{16:0}$ DMA					4.3		5.4					4.5
anteiso-$C_{17:0}$			1.6				3.5	1.2	3.8		1.9	1.6
iso-$C_{17:0}$ 3-OH					3.3	4.9		7.6	7.7		9.2	9.1
Cyclo $C_{17:0}$/$C_{18:0}$			9.4	16.2	12.1	8.1	4.0		4.8	12.3	3.0	6.3
$C_{18:1}\omega 7c$ DMA												1.1
$C_{18:1}\omega 7c/12t/9t$								1.4				2.3
$C_{18:1}\omega 9c$	**18.3**		**20.2**	**22.6**	**24.4**	**22.6**	**17.6**	2.8	**11.4**	**31.2**	5.1	**12.0**
$C_{18:1}\omega 9c$ DMA			6.6		6.3	4.4	7.3		2.6	3.1		10.3
$C_{19:0}$ CYCLO $\omega 8c$/$C_{20:0}$												
$C_{18:2}$ $\omega 6,9c$/anteiso-$C_{18:0}$	**10.8**		**22.0**	**21.1**	**20.6**	**26.8**	**19.8**	**9.7**	**24.0**	**25.7**	8.5	**12.2**
iso-$C_{19:1}$			1.5									1.0
Unknown $C_{13:835}$		5.52										
Unknown $C_{16:315}$	1.09	2.2										
Unknown $C_{18:177}$			5.1				8.9			1.9		
Unknown $C_{16:754}$			1.4									2.1

*Data from Rooney *et al.* (2011), the organism was grown on Columbia blood agar.

originally recovered from an enrichment containing xylan, it was later determined that the isolate was not utilizing this substrate. Instead, the isolate utilizes components contained within peptone and yeast extract. The novel strain M6.X2D^T appears to prefer proteinaceous materials such as amino acids or peptides that may be derived from either the diet or the host itself.

In addition to the unique 16S rRNA gene sequence, traits useful in distinguishing t strain M6.X2D^T from other species of the genus *Peptoniphilus* are shown in Tables 1 and 2, and the species description. Based on phenotypic, genotypic and chemotaxonomic evidence, this bacterium from human faecal material represents a novel species of the genus *Peptoniphilus*, for which the name *Peptoniphilus catoniae* sp. nov. is proposed.

Description of *Peptoniphilus catoniae* sp. nov.

Peptoniphilus catoniae (ca.to′ni.ae. L. gen. n. *catoniae* of Cato, in honour of Elizabeth P. Cato, a United States microbiologist for her many contributions to anaerobic microbiology).

Table 2. Phenotypic characteristics useful in the differentiation of strain M6.X2D^T from other members of the genus *Peptoniphilus*

Strains: 1, M6.X2D^T (data from this study); 2, *P. stercorisuis* DSM 27563^T (Johnson *et al.*, 2014); 3, *P. asaccharolyticus* CCUG 9988^T (Ulger-Toprak *et al.*, 2012); 4, *P. coxii* CCUG 59622^T (Citron *et al.*, 2012); 5, *P. duerdenii* WAL 18896^T (Ulger-Toprak *et al.*, 2012); 6, *P. gorbachii* CCUG 53341^T (Ulger-Toprak *et al.*, 2012); 7, *P. harei* CCUG 38491^T (Ulger-Toprak *et al.*, 2012); 8, *P. koenoeneniae* WAL 18898^T (Ulger-Toprak *et al.*, 2012); 9, *P. indolicus* CCUG 17639^T (Ulger-Toprak *et al.*, 2012); 10, *P. ivorii* CCUG 38492^T (Citron *et al.*, 2012); 11, *P. lacrimalis* CCUG 31350^T (Ulger-Toprak *et al.*, 2012); 12, *P. methioninivorax* NRRL B-23883^T (Rooney *et al.*, 2011); 13, *P. olsenii* CCUG 53342^T (Ulger-Toprak *et al.*, 2012); 14, *P. tyrrelliae* CCUG 59621^T (Citron *et al.*, 2012). +, Positive; −, negative; W, weakly positive; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Alanine arylamidase	−	+	−	−	−	−	−	−	−	−	+	W	+	−
Alkaline phosphate	−	−	−	−	−	−	W	−	+	−	−	+	+	−
Arginine dihydrolase	−	+	+	−	W	−	−	+	−	−	−	+	−	−
Glutamyl glutamic acid arylamidase	−	−	W	−	−	+	−	−	−	−	+	−	−	−
Glycine arylamidase	−	+	−	−	−	+	−	W	−	−	+	W	+	−
Indole	−	−	−	−	−	+	−	−	+	−	−	−	+	+
Leucine arylamidase	−	+	+	−	+	+	−	+	−	−	+	+	−	+
Phenylalanine arylamidase	−	+	+	−	−	W	−	+	−	−	+	W	+	−
Proline arylamindase	−	−	W	+	−	−	−	W	−	+	−	+	−	−
Pyroglutamic acid arylamidase	−	−	+	−	+	−	−	+	−	−	−	−	−	−
Serine arylamidase	−	+	+	−	−	+	−	+	−	−	+	W	+	−
Tyrosine arylamidase	−	+	+	−	−	+	−	+	−	−	+	+	+	+

Cells are Gram-stain-positive cocci, non-spore-forming and strictly anaerobic. When grown on blood agar, colonies are needlepoint in size, beige, circular with a smooth surface and raised elevation, and are non-haemolytic. Cells are typically 1.0–2.0 μm × 1.0–5.3 μm and sometimes occur as chains. The temperature range for growth is between 30 °C and 43 °C with optimal growth occurring at 37 °C. The pH range for growth is between pH 6.5 and pH 9.0 with optimal growth at pH 6.5. Salt tolerance is 0.5–2.0 % with optimal growth at 0.5 %. Catalase- and urease-negative. Nitrate is not reduced and indole is not produced. Carbohydrates are generally not utilized, with protein substrates preferred. Using the API Rapid 32A test system, acid is produced with leucyl glycine arylamidase but not with arginine dihydrolase, proline arylamidase, α-galactosidase, β-galactosidase, 6-phosphate, D-glucosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, glutamic acid decarboxylase, α-fucosidase, serine arylamidase, glutamyl glutamic acid arylamidase, alkaline phosphatase, arginine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid, mannose or raffinose. The Rapid ID 32A profile number is 0000 0400 00. Using the Biolog AN MicroPlate system, positive reactions are produced from malic acid, pyruvic acid and pyruvic acid methyl ester, and weaker reactions are obtained from D-fructose, L-fucose, palatinose, fumaric acid, α-ketobutyric acid, α-ketovaleric acid, L-methionine, L-valine plus L-aspartic acid, inosine, thymidine, uridine and 2′-deoxyadenosine. The major fatty acids are $C_{10:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:1}\omega9c$ and $C_{18:2}\omega6,9c$/anteiso-$C_{18:0}$.

The type strain M6.X2D^T (=DSM 29874^T=CCUG 66798^T) was isolated from a faecal sample of an individual from a traditional Peruvian community in the region Ica. The DNA G+C content of the type strain is 34.4 mol%.

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