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Ezakiella peruensis gen. nov., sp. nov. isolated from human fecal sample from a coastal traditional community in Peru

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Abstract

A novel Gram-stain positive, non-motile, non-sporeforming coccus-shaped, obligately anaerobic bacterium was isolated from a fecal sample of an individual residing in a traditional Peruvian community. The organism was characterized using biochemical, chemotaxonomic and phylogenetic methods. Comparative 16S rRNA gene sequence analyses and phenotypic characteristics demonstrated that the organism was biochemically and phenotypically related, but distinct, from a group of organisms referred to as the Gram-stain positive anaerobic cocci (GPAC). The major cellular fatty acids of the novel isolate were determined to be $C_{16:0}$ (18.3%), $C_{18:1\varepsilon 9c}$ (39.8%), $C_{18:2\varepsilon 6,9c}/C_{18:0\ ANTE}$ (13.2%). Fermentation end products from PYG are acetate and formate. Cell-wall peptidoglycan was found to be A4∋ (L-Lys-L-Ala-L-Glu) and the G + C content was determined to be 38.4 mol%. Based on the phenotypic, chemotaxonomic, and phylogenetic results, *Ezakiella peruensis* gen. nov., sp. nov., is now proposed. The type strain is M6.X2^T (DSM 27367^T = NBRC 109957 ^{T} = CCUG 64571^T).

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The 16S rRNA sequences of strain M6.X2^T (DSM 27367 ^{T} = NBRC 109957 ^{T} = CCUG 64571^T) have been deposited in GenBank under accession number KJ469554.

Keywords

Ezakiella peruensis gen. nov.; sp. nov.; 16S rRNA-phylogeny; taxonomy

1. Introduction

To date, the majority of studies on the human microbiome have focused on western populations, implementing the use of culture-independent methods[1-3]. These molecular inventories have provided tremendous insights into the microbial diversity and the richness of taxa present, suggesting that in the human gut, roughly 80% of the phylotypes represent uncultured bacteria[1]. In order to truly appreciate if there is a "core microbiome," individuals from a variety of geographic regions with diverse diets must also be included in these investigations [4,5]. Initial studies are now revealing that the microbiome of indigenous communities may be significantly different than those derived from "westernized" communities [6,7]. There is now a renewed interest in culture-dependent approaches to recover organisms and characterize their physiological and metabolic properties in order to better understand the ecology of these microbial communities and the role they play in both health and disease processes [8-10].

The Gram-stain positive anaerobic cocci (GPAC) are part of the commensal flora of humans and animals and are also associated with a variety of human infections [11,12]. This group of organisms has undergone extensive taxonomic changes with many former members of the *Peptostreptococcaceae* being transferred to a number of novel genera that encompass *Peptoniphilus*, *Anaerococcus*, *Finegoldia*, *Gallicola*, and *Parvimonas* [12,13]. In addition, the genera *Anaerosphaera*, *Helcococcus* and *Murdochiella* have also been described and are phylogenetically related and phenotypically similar to the aforementioned genera [14-16]. Until recently the exact relationship of this group of organisms with other close close members of *Firmicutes* was somewhat uncertain, and was reflected in their placement in the Family XI *Incertae Sedis* (order *Clostridiales*, class *Clostridia*, phylum *Firmicutes*) in the current edition of *Bergey's Manual of Systematic Bacteriology* [17,18]. However, Johnson et al., (2014)[19] recently described the family *Peptoniphilaceae* to accommodate the genera *Peptoniphilus*, *Anaerococcus*, *Anaerosphaera*, *Finegoldia*, *Gallicola*, *Helcococcus*, *Murdochiella*, and *Parvimonas*.

A study focused on a Peruvian community to examine microbial diversity of geographically remote, traditional native communities, resulted in the isolation and characterized of a Gram-positive staining, obligately anaerobic, coccus-shaped organism recovered from a fecal sample. The organism displayed phenotypic traits consistent with the GPAC but phylogenetic analysis demonstrated that it represented a novel lineage within this group. Based on the results of a polyphasic taxonomic study, we describe and propose a novel genus and species for which the name *Ezakiella peruensis* gen. nov. sp. nov. is proposed. The type strain is M6.X2^T (DSM 27367^T = NBRC 109957^T = CCUG 64571^T).

2. Materials and methods

2.1. Cultures and cultivation

Strain M6.X2^T (DSM 27367^T = NBRC 109957^T = CCUG 64571^T) was isolated from a freshly voided fecal sample obtained from a member of the Afro-Peruvian community of Cruz Verde located in Ica, Peru. The sample was collected, processed anaerobically, and transported back to the laboratory on ice for further processing. Multiple enrichments using an array of substrates were constructed and inoculated with 1 ml of fecal slurry. Strain M6.X2^T was isolated from an enrichment of Medium 2 [20] supplemented with xylan (per 100 mL distilled water): Casitone (1.0g), yeast extract (0.25g), minerals solution (A), minerals solution (B), clarified, sterile rumen fluid (20 mL), resazurin (0.0001g), sodium lactate (70% w/v) (1.0g), xylan (0.2g), cysteine HCl (0.05g), sodium bicarbonate (0.4g), distilled water (to 100 mL). Minerals solution (A) contains (per 1000 mL); K_2HP0_4 (3.0g). Minerals solution (B) contains (per 100 mL); KH_2P0_4 (3.0g), $(NH_4)_2S0_4$ (6.0g), NaCl (6.0g), $MgS0^{4\cdot}7H_20$ (0.6g), $CaCl_2$ (0.6g). The enrichment was incubated for 7 days at 37°C with a gas mix of 5% Hydrogen, 10% Carbon Dioxide, and 85% Nitrogen. After incubation, a sample of the enrichment was inoculated onto Medium 2 agar and isolates were then sub-cultured onto BD Bacto™ Brain Heart Infusion (Sparks, MD, USA) agar plates supplemented with 5% defibrinated sheep's blood until pure colonies were obtained.

2.2. Phenotypic and biochemical characterization

Unless indicated otherwise, all analyses were performed with cells grown on BD Bacto™ Brain Heart Infusion (Sparks, MD, USA) agar plates supplemented with 5% defibrinated sheep's blood or in anoxic modified peptone-yeast extract (PY) broth medium (DSMZ Medium #104 with glucose excluded) at 37°C (pH 7.2). Cells were examined with an Olympus CX41 microscope using phase contrast at 1000X magnification. For biochemical characterization, API Rapid ID 32A and API 50 CH test systems (API bioMérieux, Marcy l'Etoile, France) were used following the manufacturer's instructions except for the following modifications; both test systems were incubated anaerobically and cupules for the API 50 CH test strips were overlaid with mineral oil. Additional physiological characteristics were determined at varying temperature growth ranges (4-60 °C, in increments of ~5 °C) and pH values (5.0-9.5, in increments of 0.5 pH units). Salt tolerance was examined using different concentrations of NaCl (0-0.5 % (w/v), and 1-9% (w/v), in increments of 1%).

Optimum growth conditions were determined by monitoring the optical density using a spectrophotometer at 600 nm (Spectronic 20D, Milton Roy, DE). Growth resulting in an increase of OD_{600nm} of >0.1 was considered to indicate growth. All tests were performed in duplicate.

Metabolic end products were determined from cultures grown under anaerobic conditions in PYG broth. 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 HCL running buffer at a flow rate of 0.9 mL/min. Retention times and peak areas of fermentation products were compared to standards of acetate, butyrate, lactate, succinate, formate, and propionate.

2.3. DNA isolation and 16S rRNA gene sequencing and phylogenetic analysis

For phylogenetic analysis, DNA of strain M6.X2^T was extracted using the UltraClean® Microbial DNA Isolation Kit (MoBio Laboratories, Inc.) following manufacturer's instructions. 16S rRNA gene fragments were generated by PCR using universal primers pA (positions 8 to 28, *Escherichia coli* numbering) and pH* (1542 to 1522)[23]. The amplicon was purified using Exo-SapIt (USB Corporation) and the sequence determined using the Big Dye terminator cycle sequencing kit (ver. 3.1), with an automatic DNA sequencer (model 3100 Avant, Applied Biosystems). The closest known relatives of the new isolate based on the 16S rRNA gene sequence were determined by performing database searches using the program EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/;[24]. These sequences and those of other related strains were aligned with the sequence derived from M6.X2^T using the program ClustalW. Phylogenetic reconstructions were performed in MEGA (version 4) [25] using the neighbour-joining method [26], applying evolutionary genetic distances that had been calculated by the Kimura two-parameter model [27].

2.4. GenBank accession numbers

The 16S rRNA gene sequence for strain M6.X2^T was deposited with the EBI Sequence Database under the following accession number KJ469554.

2.5. Chemotaxonomic methods

Biomass for fatty acid analysis was collected from a plate of BHI agar amended with 5% sheep's blood after a 6 day incubation 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 [21,22]. Analysis was performed using an Agilent Technologies 6890N gas chromatograph equipped with a phenyl methyl silicone fused silica capillary column (HP-2 25m × 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. Peptidoglycan analysis was performed using the method of Hamada et al., [28] at the Biological Resource Center, National Institute of Technology and Evaluation (NBRC), Japan. The mol% G+C was determined according to the method of Mesbah et al. [29] and was carried out by the Leibniz-Institut DSMZ, Germany).

3. Results and discussion

3.1. Phenotypic and biochemical characterization

M6.X2^T is a non-motile Grain-stain positive, diplococci that is strictly anaerobic, non-spore forming and non-hemolytic. Growth on BHI blood agar plates after 6 days at 37°C colonies are small (<1mm in diameter), clear, circular, and convex. Indole is produced but catalase and urease are not while nitrate is not reduced. Using the API Rapid 32A test system, positive reactions were observed for alkaline phosphatase, arginine arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine

arylamidase, and glutamyl glutamic acid. Negative reactions were obtained for arginine dihydrolase, proline arylamidase, D-galactosidase, β-galactosidase, β-galactosidase-6 phosphate, D-glucosidase, β-glucosidase, D-arabinosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, glutamic acid decarboxylase, D-fucosidase, mannose, raffinose, serine arylamidase and urease. Using the API 50 CH test system, negative reactions were observed for glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-ßd-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-αd-mannopyranoside, methyl-αd-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-sucrose, D-trehalose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate, and potassium 5-ketogluconate. Metabolic end products from PYG were determined to be Acetate and Formate. Temperature range for growth is 30–37 °C with an optimum temperature of 37 °C. The pH range for growth is pH 7.0–8.5 with an optimum pH of pH 7.75. Growth occurs at NaCl concentrations of 0.5% (w/v) only.

3.2 Phylogenetic analysis

Phylogenetic analysis demonstrated that the organism was a member of the *Firmicutes* sharing a loose relationship with members of the GPAC (Fig. 1). These genera, *Peptoniphilus*, *Anaerococcus*, *Anaerosphaera*, *Finegoldia*, *Gallicola*, *Helcococcus*, *Murdochiella* and *Parvimonas* have recently been assigned to a newly described family *Peptoniphilaceae* [19]. The pairwise comparisons showed that strain M6.X2^T formed a loose cluster with the genera *Finegoldia* (86.7% sequence similarity), *Gallicola* (83.5% sequence similarity) and *Parvimonas* (84.4% sequence similarity). All the major groupings in the neighbor-joining tree were confirmed using the maximum parsimony program (data not shown). In addition, database searches recovered only two closely related sequences (>99% sequence similarity) corresponding to uncultured organisms, GQ016861 and GQ016867. These sequences formed a tight cluster with strain M6.X2^T but did not change the topology of the tree with respect to the other close relatives included in the analysis (data not shown). Although not recovered from fecal material they were however isolated from human skin[30].

3.3. Chemotaxonomic analysis

The fatty acid data of strain M6.X2^T represented the following: major fatty acids were $C_{16:0}$ (18.3%), $C_{18:1\varepsilon 9c}$ (39.8%) and $C_{18:2\varepsilon 6,9C/C18:0\ ANTE}$ (13.2%). The minor fatty acids were $C_{10:0}$ (7.3%), $C_{13:0\ ANTEISO}$ (3.1%), $C_{14:0}$ (3.0%), $C_{17:1\ \varepsilon 8c}$ (1.6%), $C_{18:0/17:0\ CYCLO}$ (5.2%), $C_{18:1\ \varepsilon 7c}$ (4.2%), $C_{15:0}$ (1.6%), $C_{16:1\ \varepsilon 7c}$/$C_{16:1\ \varepsilon 6c}$ (1.4%), $C_{17:0}$ (5.2%). The novel organism and its nearest relatives all produce $C_{16:0}$, $C_{18:1\varepsilon 9c}$ and $C_{18:2\varepsilon 6,9C/C18:0\ ANTE}$ as the major products. The peptidoglycan analysis revealed the presence of a A4α type with an interpeptide bridge comprising L-Lys-L-Ala-L-Glu. The DNA G+ C content of strain M6.X2^T was determined to be 38.4 mol%.

The unidentified organism from human feces was found to possess biochemical and chemotaxonomic traits consistent with organisms belonging to the GPAC but could clearly

be distinguished from its nearest phylogenetic relatives using characteristics shown in Tables 1 and 2. Although there is much discussion on the validity of describing novel genera and species on a single strain, this continues to be a common practice. We feel it is important to name this novel organism in order to allow members of the scientific community to identify additional strains or species of this novel genus. With the increasing number of cultured strains recovered from individuals from indigenous communities, a clearer picture of the ecology of these environments will emerge. Although originally recovered from an enrichments containing xylan, it was later determined that the isolate was not utilizing this substrate instead using components contained within the peptone and yeast extract. For instance, the novel strain M6.X2^T appears to prefer proteinaceous materials such as amino acids or peptides that can be derived from either the diet or the host itself.

Based on the biochemical, chemotaxonomic and phylogenetic data presented, we consider the coccus-shaped organism from human feces represents a novel genus within the GPAC for which the name *Ezakiella peruensis* gen. nov., sp. nov. is proposed.

4. Description of *Ezakiella* gen. nov.

Ezakiella (E.za.ki.el'la N.L. fem. dim. *Ezakiella* named after the Japanese microbiologist Takayuki Ezaki who has contributed immensely to the taxonomy of the anaerobic Gram-stain positive cocci group of bacteria).

Cells are Gram-stain positive, non-motile, cocci that are strictly anaerobic. Catalase and urease negative. The predominant fatty acids are $C_{16:0}$, $C_{18:1\ \omega 9c}$, $C_{18:2\ \omega 6,9C/18:0\ ante}$. Cell-wall peptidoglycan is A4∋ (L-Lys-L-Ala-L-Glu). The DNA G+ C content of the type strain of the type species is 38.4 mol%. The genus *Ezakiella* is a member of the phylum *Firmicutes* within the family *Peptoniphilaceae*. The type species is *Ezakiella peruensis*.

Description of *Ezakiella peruensis* sp. nov.

Ezakiella peruensis (pe.ru.en'sis. N.L. fem. adj. *peruensis* pe.ru.en'sis N. L. gen. n. pertaining to the country of Peru from where the organism was first isolated). Displays the following properties in addition to those given in the genus description. After 6 days of growth on BHI Blood agar plates colonies are small (<1mm in diameter), clear, circular, and convex. Catalase and urease negative. Nitrate is not reduced and indole is produced. The API Rapid ID 32An test system, positive reactions are obtained for alkaline phosphatase, arginine arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, pyroglutamic acid arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, and glutamyl glutamic acid. Negative reactions are obtained for arginine dihydrolase, proline arylamidase, D-galactosidase, β-galactosidase, β-galactosidase-6 phosphate, D-glucosidase, β-glucosidase, D-arabinosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, glutamic acid decarboxylase, D-fucosidase, mannose, raffinose, serine arylamidase and urease. Using the API 50 CH test system under anaerobic conditions no positive reactions are observed. Metabolic end products from PYG are acetate and formate.

The type strain M6.X2^T (DSM 27367^T = NBRC 109957^T = CCUG 64571^T) was isolated from a fecal sample of an individual from a traditional Peruvian community in the region Ica.

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Abbreviations

BD	Becton, Dickinson and Company
CCUG	Culture Collection-University of Goteborg
GPAC	Gram-Positive Anaerobic Cocci
HMP	Human Microbiome Project
NBRC	NITE Biological Resource Center
PY	peptone-yeast extract

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Highlights

This work demonstrates the importance of cultivation studies to augment the large number of molecular-based studies on the human gut microbiome. The use of these methods will help further our understanding of this important ecosystem. Any “yet-to-becultivated” organisms remain to be identified and characterized.

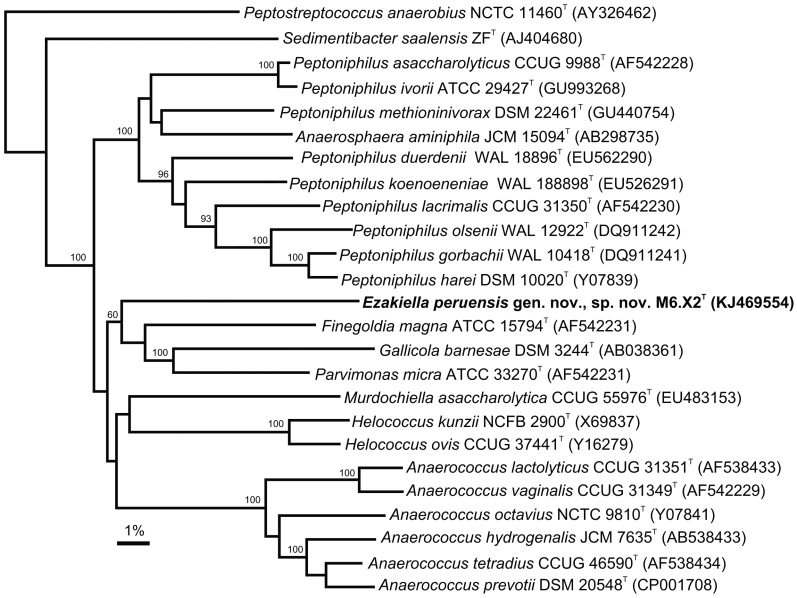

Fig. 1.

Phylogenetic tree of 16S rRNA gene sequences indicating the position of *Ezakiella peruensis* gen. nov. sp. nov within members of related genera within the phylum *Firmicutes*. The tree was constructed using the neighbour-joining algorithm from MEGA (version 4) with *Peptostreptococcus anaerobius* as the outgroup. Bootstrap values (%) were obtained with 1000 replicates and are displayed on their relative branches.

Table 1

Fatty acid profiles of strain M6.X2^T and close relatives.

Fatty Acid [a]	M6.X2^T	*Anaerococcus prevotii* CCUG 41932^T	*Anaerosphaera aminiphila* JCM 15094^T	*Finegoldia magna* CCUG 17636^T	*Murdochiella asaccharolytica* CCUG 55976^T	*Helcococcus kunzii* CCUG 32213^T	*Parvimonas micra* CCUG 46357^T	*Peptoniphilus asaccharolyticus* CCUG 9988^T
$C_{10:0}$	7.3			9.4				
$C_{12:0}$				4.8				12.0
anteiso-$C_{13:0}$	3.1							
$C_{14:0}$	3.0	2.0	2.0	5.9	**11.0**	2.5	1.6	5.4
$C_{15:0}$	1.6							
iso-$C_{15:0}$								2.6
$C_{16:0}$	**18.3**	**17.1**	9.4	**17.6**	**34.0**	**30.0**	**13.4**	14.4
$C_{16:0\ alde}$			6.8	6.4				
$C_{16:0\ DMA}$			6.5					
$C_{16:1}$ ω7c/ $C_{16:1}\omega_{6c}$	1.4							
$C_{16:1}$ ω7c			2.0			2.8	2.0	3.9
$C_{16:1}$ ω9c			7.4					
$C_{17:1\omega}$8c	1.6		14.3					
$C_{17:1\omega}$9c			7.1					
iso-$C_{17:1\omega}$5c		3.9						3.0
anteiso-$C_{17:0}$		1.7		4.5				1.6
$C_{17:0\ DMA}$								
iso-$C_{17:1}$/$C_{16:0\ DMA}$				**18.2**				
Cyclo $C_{17:0}$/ $C_{18:0}$	5.2							
$C_{18:0}$		**11.5**				**16.0**	6.8	9.4
$C_{18:0\ ald}$			4.4					
$C_{18:0\ DMA}$			2.0					
$C_{18:1}$ ω_{7c}	4.2		6.9					
$C_{18:1}$ ω_7 DMA			12.2					
$C_{18:1}$ ω9c	**39.8**	**19.3**	2.0	3.6	**54.0**	**19.3**	**15.5**	**20.2**
$C_{18:2}$ $\omega_{6,9c}$/ $C_{18:0\ ANTE}$	**13.2**	**27.0**		5.6		**29.4**	**58.3**	**22.0**
$C_{18:1}$ ω9c DMA			6.4	**11.1**				6.6
iso-$C_{19:1}$		2.0						1.5
Unknown $C_{18:177}$		6.9		**13.1**				5.1

Fatty Acid [a]	**M6.X2^T**	***Anaerococcus prevotii* CCUG 41932^T**	***Anaerosphaera aminiphila* JCM 15094^T**	***Finegoldia magna* CCUG 17636^T**	***Murdochiella asaccharolytica* CCUG 55976^T**	***Helcococcus kunzii* CCUG 32213^T**	***Parvimonas micra* CCUG 46357^T**	***Peptoniphilus asaccharolyticus* CCUG 9988^T**
$C_{20:4}\omega_{6,9,12,15c}$	1.4							

[a] Predominant products are shown in bold, values below 1% are not shown. Profiles for *Gallicola* have not been performed.

Table 2

Morphological, biochemical and chemotaxonomic properties that are useful in the differentiation of strain M6.X2^T and the type species of its close relatives.

Characteristic	*Ezakiella*	*Anaerococcus*	*Anaerosphaera*	*Finegoldia*	*Gallicola*	*Helcococcus*	*Murdochiella*	*Parvimonas*	*Peptoniphilus*
Spore formation or thermotolerant cell	−	−	+	−	−	−	−	−	−
Fermentation End Product (PYG)	A, F	A, B L,	A, B	A	A, B	L, A	L	A	A, B
Metabolism	Obligate anaerobe	Obligate anaerobe	Obligate anaerobe	Obligate anaerobe	Obligate anaerobe	Facultative anaerobe	Obligate anaerobe	Obligate anaerobe	Obligate anaerobe
Production of Indole	+	−	−	−	w	−	+	−	+
Fermentation of: Mannose Raffinose	− −	− w	− −	− −	− −	+ +	w w	− −	− −
Activity of: Alkaline phosphatase Arginine arylamidase Histine arylamidase Leucine arylamidase Proline arylamidase Phenylalanine arylamidase Pyroglutamic acid arylamidase	+ + + + − + +	− + + w − w +	nd nd nd nd nd nd nd	+ + w + − − +	− − − − − − −	− − − − − + −	− + + + + + −	+ + + + + + +	− + + − − − −
API rapid 32A profile	0000657707	5444017305	nd	2000456605	nd	0517004020	2006272705	2000477707	2000210001
Cell-wall murein	Lys	Lys, D-Glu	Lys	Lys, D-Asp	D-Asp	D-Asp	ND	Lys	Orn, D-Glu
DNA G + C content (mol%)	38.4	30-35	32.5	32-34	27-34	ND	ND	27-28	30-34
Source	Human feces	Human intestine, clinical material, vaginal discharges, ovarian, peritoneal and other bodily abscesses, skin,	Methanogenic cattle manure reactor	Vagina, oral cavity and other bodily abscesses	Chicken feces	Skin, human clinical material, sheep	Human clinical material	Oral cavity and human abscesses	Human intestine and clinical material, vaginal discharges, ovarian, peritoneal and other bodily abscesses, cattle

Characteristic	*Ezakiella*	*Anaerococcus*	*Anaerosphaera*	*Finegoldia*	*Gallicola*	*Helcococcus*	*Murdochiella*	*Parvimonas*	*Peptoniphilus*
		and nasal passage							mastitis and swine manure

Data: *Ezakiella peruensis* (M6.X2^{T}), this study; *Anaerococcus* [31]; *Anaerosphaera*[14]; *Finegoldia*[32]; *Gallicola*[31]; *Helcococcus*[15]; *Murdochiella*[16]; *Parvimonas*[33]; *Peptoniphilus*[31]. Additional data obtained from de Vos et al., (2009) and the CCUG web site. (www.ccug.se)

+, positive; -, negative; nd, no data; A, acetate; B, Butyrate, F, Formate, L, Lactate; m-Dpm, *meso*-diaminopimelic acid