***Enterococcus villorum* sp. nov., an enteroadherent bacterium associated with diarrhoea in piglets**

M. Vancanneyt,¹ C. Snauwaert,¹ I. Cleenwerck,¹ M. Baele,³
P. Descheemaeker,^{4†} H. Goossens,⁴ B. Pot,^{1‡} P. Vandamme,² J. Swings,^{1,2}
F. Haesebrouck³ and L. A. Devriese³

Author for correspondence: M. Vancanneyt. Tel: +32 9 2645115. Fax: +32 9 2645092.
e-mail: Marc.Vancanneyt@rug.ac.be

^{1,2,3} BCCM/LMG Bacteria Collection¹, Laboratory of Microbiology, Faculty of Sciences² and Laboratory of Bacteriology, Faculty of Veterinary Medicine³, University of Gent, Gent, Belgium

⁴ Department of Medical Microbiology, University Hospital, UIA, Antwerp, Belgium

The taxonomic positions of five enteroadherent bacterial pig isolates, showing phenotypic characteristics most similar to those of *Enterococcus durans* and *Enterococcus hirae*, were investigated in a polyphasic study that included 16S rDNA sequence analysis, DNA–DNA hybridizations, DNA base-ratio determinations, whole-cell protein fingerprinting, D11344-primed PCR typing and an extensive examination of phenotypic properties. The results demonstrated that the organisms represent a new species in the *Enterococcus faecium* species group, for which the name *Enterococcus villorum* sp. nov. is proposed. The type strain is LMG 12287^T (= CCM 4887^T).

Keywords: *Enterococcus villorum* sp. nov., identification, polyphasic taxonomy, adhesion, pig

INTRODUCTION

The enterococci belong phylogenetically to the clostridial subdivision of the Gram-positive bacteria. They form a distinct cluster, with *Vagococcus*, *Tetragenococcus* and *Carnobacterium* as their closest neighbours (Collins *et al.*, 1989; Aguirre & Collins, 1992; Devriese *et al.*, 1993; Devriese & Pot, 1995; Franz *et al.*, 1999). The phenotypic description of the genus *Enterococcus* is vague, as there are no characters that distinguish them unequivocally from other Gram-positive, catalase-negative, coccus-shaped bacteria (Devriese *et al.*, 1993; Devriese & Pot, 1995). Currently, almost 20 species are assigned to the genus, which is subdivided in at least four species groups based on 16S rRNA gene sequence similarity (Williams *et al.*, 1991; Devriese & Pot, 1995; Teixeira *et al.*, 1996; Stiles & Holzapfel, 1997; De Vaux *et al.*, 1998).

The *Enterococcus faecium* species group currently contains four species: *E. faecium*, *Enterococcus durans*, *Enterococcus hirae* and *Enterococcus mundtii*. The history of the species *E. faecium*, *E. durans* and *E. hirae*

cannot be considered separately. When first described, the species *E. faecium* and *E. durans* were classified in the genus *Streptococcus* (Orla-Jensen, 1919; Sherman & Wing, 1937). In 1970, Kalina proposed to transfer [*Streptococcus*] *faecium* to the genus *Enterococcus* and to reclassify [*Streptococcus*] *durans* as *E. faecium* subsp. *durans*. Both propositions of Kalina (1970) were not generally accepted, although several other authors confirmed that, on the basis of phenotypic similarity, [*S.*] *faecium* and [*S.*] *durans* represent a single taxon (Deibel & Seeley, 1974; Jones, 1978). Only after extended nucleic acid studies was the genus *Enterococcus* officially recognized and it initially comprised two species, the type species *Enterococcus faecalis* and *E. faecium* (Schleifer & Kilpper-Bälz, 1984). Shortly afterwards, [*S.*] *durans* was reclassified in the genus *Enterococcus* as *E. durans*, based on its low DNA–DNA homology with *E. faecium* and few phenotypic differences (Collins *et al.*, 1984). One year later, it was demonstrated that strains with biochemical properties intermediate between those of *E. faecium* and *E. durans* constituted a separate genomic species and these were named *E. hirae* (Farrow & Collins, 1985). The most recently described member of the *E. faecium* species group is *E. mundtii*, containing yellow-pigmented non-motile strains (Collins *et al.*, 1986).

Members of the *E. faecium* species group showing typical adhesion to the apical surface of the enterocytes

[†] **Present address:** Laboratory of Microbiology, AZ St.-Jan AV, Brugge, Belgium.

[‡] **Present address:** Yakult Belgium sa/nv, Brussels, Belgium.

The EMBL accession number for the 16S rDNA sequence of *Enterococcus villorum* LMG 12287^T is AJ271329.

of the small intestine of animals have been described since the mid 1980s. They have been implicated as possible causes of diarrhoea in the young of different mammal host species (see Discussion). Such strains often remained unidentified or were classified as *E. durans* or *E. hirae*.

In the present paper, a polyphasic study was performed on a collection of five isolates, all originating from pigs. Two were originally identified as *E. durans* from cases of diarrhoea in piglets in Canada and Korea. A third strain was isolated in the USA from the jejunum of a nursery pig. Preliminary analyses of these three isolates revealed high phenotypic similarity to two pig isolates received from R. R. Facklam (Centers for Disease Control and Prevention, Atlanta, GA, USA); all five isolates were tentatively classified as *E. hirae*-like.

A polyphasic taxonomic study indicated that the five pig isolates could not be assigned to a known *Enterococcus* species, but constituted a separate but homogeneous taxon. All data demonstrated that they represented a new species, for which the name *Enterococcus villorum* sp. nov. is proposed.

METHODS

Bacterial strains and growth conditions. The sources of the isolates and reference strains studied are listed in Table 1. All strains were grown and maintained on MRS agar (Oxoid) and incubated aerobically at 37 °C, unless indicated otherwise. Bacteriological purity was checked by plating and examining living and Gram-stained cells.

PAGE of whole-cell proteins. After incubation of cells for 24 h, whole-cell protein extracts were prepared and SDS-PAGE was performed as described by Pot *et al.* (1994). Densitometric analysis, normalization and interpolation of the protein profiles and numerical analysis were performed by using the GELCOMP software package, versions 3.1 and 4.0 (Applied Maths).

D11344-primed PCR analysis. DNA was prepared following the method described by Pitcher *et al.* (1989). PCR amplification using the oligonucleotide D11344 as primer and subsequent electrophoresis were performed as described previously (Descheemaeker *et al.*, 1997). Conversion, normalization and numerical analysis of the fingerprints were performed by using the GELCOMP software package.

16S rDNA sequence analysis. Genomic DNA of *E. villorum* LMG 12287^T was extracted by using the Puregene DNA isolation kit (Gentra Systems) according to the protocol supplied by the manufacturer with the following modification: proteinase K (200 µg ml⁻¹; Merck) was added during lysis of the cells. A fragment of the 16S rDNA gene (corresponding to positions 10–1541 in the *Escherichia coli* numbering system) was amplified by PCR using the conserved primers MH1 (5'-AGTTTGATCCTGGCTCAG-3') and pH (5'-AAGGAGGTGATCCAGCCGCA-3'). The PCR product was purified using a Qiaquick PCR purification kit (Qiagen), according to the manufacturer's instructions. Sequencing was performed using an Applied Biosystems 377 DNA Sequencer and the protocols of the manufacturer (Perkin-Elmer) with the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction kit. The eight sequencing primers used are listed in Coenye *et al.* (1999). Sequence assembly was performed by using the program AUTOASSEMBLER (Perkin-Elmer).

Phylogenetic analysis was performed using the software

Table 1 Strains studied

Culture collections are abbreviated as: ATCC, American Type Culture Collection, Manassas, VA, USA; CCM, Czech Collection of Microorganisms, Brno, Czech Republic; CDC, Centers for Disease Control or Communicable Disease Center, US Public Health Service, Atlanta, GA, USA; LMG, BCCM/LMG Culture Collection Laboratorium voor Microbiologie, University of Gent, Gent, Belgium; NCFB, National Collection of Food Bacteria, Agricultural and Food Research Council Institute of Food Research, Aberdeen, UK.

Taxon	Strain	Other strain designations	Source and place of isolation
<i>Enterococcus avium</i>	LMG 10744 ^T	ATCC 14025 ^T	Human, faeces
<i>Enterococcus durans</i>	LMG 10746 ^T	ATCC 19432 ^T	Dried milk
<i>Enterococcus durans</i>	LMG 12283	SP 2	Chicken, intestine; Belgium
<i>Enterococcus durans</i>	LMG 12903	NCFB 498	Cheddar cheese
<i>Enterococcus durans</i>	LMG 14202	GBg22	Herring, meat; Belgium
<i>Enterococcus faecium</i>	LMG 11423 ^T	NCFB 942 ^T	
<i>Enterococcus gallinarum</i>	LMG 13129 ^T	ATCC 49573 ^T	Chicken, intestine
<i>Enterococcus hirae</i>	LMG 6399 ^T	ATCC 8043 ^T	
<i>Enterococcus hirae</i>	LMG 11425	NCFB 1648	
<i>Enterococcus hirae</i>	LMG 11492	NCFB 2683	Chicken, crop
<i>Enterococcus hirae</i>	LMG 14489	94/290	Parrot, spleen; Belgium
<i>Enterococcus mundtii</i>	LMG 10748 ^T	ATCC 43186 ^T	Soil
<i>Enterococcus villorum</i>	LMG 12287 ^T	88-5474 ^T , CCM 4887 ^T	Pig, intestine; Canada
<i>Enterococcus villorum</i>	LMG 16296	CDC 1391-83	Pig; USA
<i>Enterococcus villorum</i>	LMG 17496	St-Paul 4204	Pig, intestine; USA
<i>Enterococcus villorum</i>	LMG 19177	CDC 1390-83	Pig; USA
<i>Enterococcus villorum</i>	LMG 19179	Strain SNUVP	Pig, intestine; Korea

Fig. 1. Protein profiles of *Enterococcus* strains and the corresponding dendrogram derived from the unweighted pair group average linkage of correlation coefficients r (expressed for convenience as percentages).

package GENECOMPAR (Applied Maths) after including the consensus sequence in an alignment of small ribosomal subunit sequences collected from the international nucleotide sequence library EMBL. This alignment was calculated pairwise using an open gap penalty of 100% and a unit gap penalty of 0%. A similarity matrix was created by homology calculation with a gap penalty of 0%; unknown bases were discarded. The resulting tree was constructed using the neighbour-joining method.

DNA base compositions. Cells were cultivated in MRS broth (Oxoid) for 24 h at 37 °C. DNA was extracted from 2.5 g wet weight of cells using the protocol described by Marmur (1961) with the following modifications: (i) 1 h before harvesting, penicillin G (Sigma) was added to a final concentration of 250 mg l⁻¹; (ii) cells suspended in Tris/HCl buffer were treated with lysozyme (8 mg ml⁻¹) overnight before addition of SDS; and (iii) lysed cells were treated with proteinase K (360 mg l⁻¹; Merck) for 2 h at 37 °C. For determination of the DNA base composition, DNA was enzymically degraded into nucleosides as described by Mesbah *et al.* (1989). The nucleoside mixture obtained was then separated by HPLC using a Waters Symmetry Shield C8 column thermostatted at 37 °C. The solvent was 0.02 M (NH₄)H₂PO₄ (pH 4.0) with 1.5% acetonitrile. Non-methylated lambda phage DNA (Sigma) was used as the calibration reference.

DNA–DNA hybridization experiments. High-molecular-mass native DNA was prepared as described above for determination of the DNA base composition. DNA–DNA hybridizations were performed using a modification of the microplate method described by Ezaki *et al.* (1989), using a HTS7000 Bio Assay Reader (Perkin-Elmer) for the

fluorescence measurements. Biotinylated DNA was hybridized with single-stranded unlabelled DNA, non-covalently bound to microplate wells. Hybridizations were performed at 34 °C in hybridization mixture (2 × SSC, 5 × Denhardt's solution, 2.5% dextran sulfate, 50% formamide, 100 µg denatured salmon sperm DNA ml⁻¹, 1250 ng biotinylated probe DNA ml⁻¹).

Phenotypic analyses. Growth tests and biochemical activities were carried out as described by Devriese *et al.* (1997). The galleries API 20 STREP, API 50 CH and rapid ID32 STREP (bioMérieux) were used and the BBL CRYSTAL Gram-positive kit (Becton Dickinson) was used as an additional test system. Susceptibility to antibiotics was evaluated on Isosensitest medium (Oxoid) using Sensitab tablets (Rosco). Tests were carried out and interpreted according to the instructions of the manufacturers.

RESULTS

PAGE of whole-cell proteins

Duplicate protein extracts were prepared to check the reproducibility of the growth conditions and the preparation of the extracts. The correlation coefficient between duplicate protein patterns was $r \geq 0.95$.

The whole-cell protein profiles of the *E. villorum* strains were initially compared with patterns of more than 600 enterococcal strains, representing all currently described *Enterococcus* species, and formed a separate cluster (Pot & Janssens, 1993; data not shown). In Fig. 1, a dendrogram is shown that was obtained after

Fig. 2. D11344-primed PCR patterns of *Enterococcus* strains and the corresponding dendrogram derived from the unweighted pair group average linkage of correlation coefficients r (expressed for convenience as percentages).

average-linkage cluster analysis of *E. villorum* strains and reference strains of *E. durans*, *E. hirae* and other members of the *E. faecium* species group. For comparison, we also included the type strain of *Enterococcus avium*, a member of the *E. avium* species group, and *Enterococcus gallinarum*, a member of the *E. gallinarum* species group. The *E. villorum* strains grouped in a single cluster at a level of $r \geq 0.89$, clearly separated from clusters containing strains of *E. durans* ($r \geq 0.88$) and *E. hirae* ($r \geq 0.84$). Other reference strains constituted distinct branches at a level of $r \leq 0.83$.

Visual inspection of the electrophoregrams (Fig. 1) revealed almost identical electrophoretic patterns within each cluster.

D11344-primed PCR analysis

The r value for reproducibility of the PCR assay and the running conditions, as estimated by the analysis of duplicate DNA extracts of several strains, was higher than 0.92.

Numerical analysis of the DNA band patterns is presented in Fig. 2 and showed that *E. villorum* strains formed a single cluster, well separated from the species-specific clusters obtained for the reference taxa.

Visual comparison of the band patterns (Fig. 2) within each of the clusters revealed only minor differences between most strains.

16S rDNA sequence analysis

The 16S rDNA gene of *E. villorum* LMG 12287^T showed highest similarities to sequences of reference strains of the *E. faecium* species group (99.1–98.8%). Somewhat lower values were observed to members of

Fig. 3. Distance matrix tree showing the phylogenetic relationships of *Enterococcus* species based on 16S rDNA sequence comparisons. *Enterococcus seriolicida* was used as the outgroup and bootstrap probability values are indicated at the branch-points (100 trees resampled). Accession numbers are given, together with strain designations where known.

the *E. avium* species group (98.4–98.1%), the *E. gallinarum* species group (98.0–97.8%), *Enterococcus dispar* (97.6%) and *Enterococcus saccharolyticus* (97.5%). Values lower than 97% were found with other described species. A phylogenetic tree is presented in Fig. 3.

Table 2 Percentage DNA–DNA binding values of *E. villorum* strains and related *Enterococcus* species

Source of DNA	1	2	3	4	5	6	7	8
1. <i>E. villorum</i> LMG 12287 ^T	100							
2. <i>E. villorum</i> LMG 17496	99	100						
3. <i>E. durans</i> LMG 10746 ^T	31	27	100					
4. <i>E. faecium</i> LMG 11423 ^T	27	28	36	100				
5. <i>E. hirae</i> LMG 6399 ^T	37	38	26	30	100			
6. <i>E. mundtii</i> LMG 10748 ^T	29	31	20	29	27	100		
7. <i>E. avium</i> LMG 10744 ^T	14	14	12	13	12	11	100	
8. <i>E. gallinarum</i> LMG 13129 ^T	15	17	16	14	14	14	13	100

Table 3 Characteristics that differentiate *E. villorum* from members of the *E. faecium* species group

Data for taxa other than *E. villorum* were obtained from Devriese & Pot (1995) and Knight & Shlaes (1986). Characters are scored as: +, positive; D, strain-dependent; D+, usually positive; D–, usually negative; –, negative. ND, Not determined.

Characteristic	<i>E. villorum</i>	<i>E. hirae</i>	<i>E. durans</i>	<i>E. faecium</i>	<i>E. mundtii</i>
Yellow pigmentation	–	–	–	–	+
Clotting of skimmed milk	–	–	D+	D	ND
β -Mannosidase	–	D+	D+	D+	ND
Susceptibility to tobramycin	+	–	+	ND	ND
Production of acid from:					
L-Arabinose	–	–	–	+	+
Mannitol	–	–	–	D+	+
Methyl β -D-glucopyranoside*	–	+	+	ND	ND
Raffinose	–	D–	D–	–†	D+
Sucrose	–	+	D	D+	+
D-Xylose	D+	–	–	D–	+

* Used at 0.05% instead of the usual 1% in phenol red broth.

† *E. faecium* strains from poultry are usually positive for production of acid from raffinose.

DNA base composition

The DNA G + C content of strains LMG 12287^T and LMG 17496 was 35.3 and 35.1 mol%, respectively.

DNA–DNA hybridization experiments

Results are summarized in Table 2. The level of DNA–DNA binding between *E. villorum* strains LMG 12287^T and LMG 17496 was 99%. Homology levels of 27–38% were observed between *E. villorum* strains and members of the *E. faecium* species group. The highest binding values were found with *E. hirae* (37–38%). Significantly lower values were observed with members of the *E. avium* and *E. gallinarum* species groups (14–17%).

Phenotypic analyses

Colonies were enterococcal-like and they showed alpha haemolysis on blood agar. The strains were not motile and produced uniform turbidity in broth

cultures. They grew on azide-containing enterococcal selective media, but colonies developed more slowly than is commonly seen with related enterococci of the *E. faecium* species group. The strains grew in 6.5% NaCl broth and caused blackening of bile/aesculin agar.

From a large set of phenotypic tests, only three differentiated all *E. villorum* strains from their closest taxonomic neighbour, *E. hirae*: inability to produce acid from methyl β -D-glucopyranoside and sucrose and susceptibility to tobramycin. Characteristics that differentiate *E. villorum* from other taxa of the *E. faecium* species group are shown in Table 3. A detailed phenotypic description of *E. villorum* is given below.

DISCUSSION

A polyphasic taxonomic study was performed to clarify the taxonomic position of five *E. hirae*-like isolates. The results of the present study allowed us to assign all five strains to a new species, for which we propose the name *E. villorum* sp. nov.

Taxonomic position of *E. villorum*

Comparison of the 16S rDNA sequence of strain LMG 12287^T with those of other species of the genus *Enterococcus* (Fig. 3) indicated that the closest relatives are members of the *E. faecium* species group (99.1–98.8%) and confirmed the high phenotypic similarity to the species *E. durans* and *E. hirae*. DNA–DNA hybridizations were performed between *E. villorum* LMG 12287^T and LMG 17496 and the type strains of all members of the *E. faecium* species group (Table 2). For comparison, the type strains of *E. avium* and *E. gallinarum* were also included. Among the *E. villorum* strains, DNA-binding values of 99% were observed. No significant DNA relatedness was measured between strains of the latter taxon and the type strains of other *Enterococcus* species. It should be noted, however, that considerably higher binding values were observed with members of the *E. faecium* species group (27–38%) than with representative strains of the *E. avium* species group (14%) and *E. gallinarum* species group (15–17%). A highest binding value of 37–38% with *E. hirae* confirmed the high phenotypic relatedness to the latter taxon.

Differentiation of *E. villorum* from other *Enterococcus* species

Both whole-cell protein electrophoresis and D11344-primed PCR analysis indicate that *E. villorum* strains form a homogeneous group that can be differentiated easily from other *Enterococcus* species (Figs 1 and 2). Both methodologies have been described for species-level differentiation of enterococci (Descheemaeker *et al.*, 1997).

Using biochemical tests (Table 3), *E. villorum* can be differentiated from *E. hirae* and *E. durans* by its inability to acidify methyl β -D-glucopyranoside and by the ability of most strains to produce acid from D-xylose. Except for its sensitivity to tobramycin and lack of activity on sucrose, no other features tested allowed the differentiation of the new taxon from *E. hirae*. Differentiation from *E. durans* is even more difficult. Clotting of skimmed milk and β -mannosidase are useful additional tests, but they are not really discriminative. Like *E. durans*, *E. villorum* is susceptible to tobramycin (Knight & Shlaes, 1986). Other characteristics useful in the differentiation of *E. villorum* from members of the *E. faecium* species group are presented in Table 3.

Source of isolation and pathogenicity

The *E. villorum* strains were all isolated from pigs and originate from three different countries: Canada (LMG 12287^T), Korea (LMG 19179) and United States (LMG 16296, LMG 17496 and LMG 19177). Two of them (LMG 12287^T and LMG 19179) were associated with neonatal diarrhoea in piglets (Drolet *et al.*, 1990; Cheon & Chae, 1996); mortality was negligible. In both cases, the most consistent and

predominant histological feature was the extensive colonization of the mucosal surface of the small intestine. Colonization extended from the tips to the bases of the villi. In both cases, the pathogenesis of diarrhoea remained unknown and was not associated with enterotoxin production or substantial mucosal injury. It was suggested that a decreased activity of brush border digestive enzymes such as lactase and alkaline phosphatase interferes with digestion and absorption of the brush border (Drolet *et al.*, 1990; Cheon & Chae, 1996). A third strain, LMG 17496, was isolated in the Minnesota Veterinary Diagnostic Laboratory of the University of Minnesota at St Paul (MN, USA) from a nursery pig with a jejunum that was colonized with Gram-positive cocci (C. Lindeman, personal communication). Two other porcine isolates, for which no detailed information on origin was provided, were obtained from the CDC (LMG 16296 and LMG 19177).

Enterococci that show typical adhesion to the apical surface of the enterocytes of the small intestine of animals have been described as possible causes of diarrhoea in the young of different mammal host species: foals (Tzipori *et al.*, 1984), infant rats (Etheridge *et al.*, 1988; Etheridge & Vonderfecht, 1992; Hoover *et al.*, 1985), piglets before weaning (Drolet *et al.*, 1990; Cheon & Chae, 1996), calves (Rogers *et al.*, 1992) and dog pups (Collins *et al.*, 1988). One report concerned a similar condition in adult dogs (Jergens *et al.*, 1991) and another in an adult cat (Hélie & Higgins, 1999). In most of these studies, the enteropathogen was presumptively identified as *E. durans* (Tzipori *et al.*, 1984; Drolet *et al.*, 1990; Cheon & Chae, 1996; Rogers *et al.*, 1992; Collins *et al.*, 1988). According to Devriese & Pot (1995), however, *E. durans* is found in domestic animals mainly in the intestines of some preruminant calves (Devriese *et al.*, 1992) and in young chicks (Devriese *et al.*, 1991), and no reports are available on the pathogenicity of the species. Several animal pathogenic strains, originally identified as *E. durans*, were reclassified as *E. hirae* (Devriese & Haesebrouck, 1991). *E. hirae* is found in the gut of all important domestic animal species (Devriese *et al.*, 1987).

Whether or not the enteroadherent enterococci reported in these animal hosts belong to *E. villorum* remains to be determined. The characteristics that have been reported for the rat strains differ from those of *E. villorum*. The strains from calves and dogs have not been characterized in detail in the literature and were not available for study.

Description of *Enterococcus villorum* sp. nov.

Enterococcus villorum (vil.lo'rum. L. n. *villus* rough hair, anatomical term for flocculate structures in the small intestine; gen. pl. *villorum*).

Strains are Gram-positive, non-sporulating and non-motile cocci. Colonies grow on azide-containing enterococcal selective media, on bile/aesculin agar and

in 6.5% NaCl broth. Colonies on Slanetz–Bartley medium are pink and develop slowly. All strains are alpha-haemolytic.

All strains react positively in the following tests: Voges–Proskauer, β -glucosidase, pyrrolidonylarylamidase, leucine arylamidase, arginine dihydrolase, glycytryptophan arylamidase, hydrolysis of aesculin, 4-methylumbelliferyl (MU) β -D-glucoside, 4-MU *N*-acetyl β -D-glucosaminide, L-phenylalanine 7-amido-4-methylcoumarin (AMC), L-pyrroglutamic acid AMC, L-tryptophan AMC, *p*-nitrophenyl β -D-glucoside, *p*-nitrophenyl β -D-cellobioside, *o*-nitrophenyl β -D-galactoside and *p*-nitrophenyl α -D-galactoside. All strains produce acid from amygdalin, arbutin, cellobiose, glycerol (weak and only visible after 2 d), D-glucose, *N*-acetylglucosamine, D-fructose, galactose, β -gentiobiose, lactose, maltose, D-mannose, melibiose, ribose, salicin and trehalose. All strains are negative in the amylase plate test, in tests for catalase, hippurate hydrolysis, alkaline phosphatase, β -glucuronidase, β -mannosidase, alanine-phenylalanine-proline arylamidase, hydrolysis of L-valine AMC, 4-MU α -D-glucoside, L-arginine AMC, 4-MU phosphate, 4-MU β -D-glucuronide, L-isoleucine AMC, *p*-nitrophenyl phosphate, proline- and leucine-*p*-nitroanilide, urease and acid production from adonitol, D- and L-arabitol, D- and L-arabinose, D-cyclodextrin, 2- and 5-keto-gluconate, dulcitol, erythritol, D- and L-fucose, α -methyl D-glucoside, methyl β -D-glucopyranoside, gluconate, glycogen, inositol, inulin, D-lyxose, maltotriose, mannitol, α -methyl D-mannoside, melezitose, pullulan, raffinose, rhamnose, sorbitol, L-sorbose, sucrose, D-tagatose, xylitol, L-xylose and β -methyl D-xyloside. Four strains produce acid from D-xylose and three strains react positively in the *p*-nitrophenyl α -D-maltoside test. These tests were the only variable reactions noted. All strains are susceptible to the aminoglycoside antibiotic tobramycin.

The organisms examined were isolated from pigs and originate from three different countries. Adhesion to enterocytes is a prominent characteristic of at least three of the strains studied. The type strain is LMG 12287^T, which was isolated from the gut of a piglet in Canada and was associated with neonatal diarrhoea. The DNA G + C content is 35.1–35.3 mol %.

All strains have been deposited in the BCCM/LMG Bacteria Collection (Laboratorium voor Microbiologie Gent, Gent, Belgium) and the type strain has been deposited in the Czech Collection of Microorganisms (Masaryk University, Brno, Czech Republic).

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