Erratum to: Two novel species *Enterococcus lemanii* sp. nov. and *Enterococcus eurekensis* sp. nov., isolated from a swine-manure storage pit

Michael A. Cotta · Terence R. Whitehead ·
Enevold Falsen · Edward Moore · Paul A. Lawson

Published online: 17 April 2013
© Springer Science+Business Media Dordrecht (outside the USA) 2013

Abstract A polyphasic taxonomic study using morphological, biochemical, chemotaxonomic and molecular genetic methods was performed on six strains of an unknown Gram-positive, nonspore-forming, facultative anaerobic coccus-shaped bacterium isolated from a swine-manure storage pit. On the basis of 16S rRNA, RNA polymerase-subunit (*rpoA*), and the 60-kilodalton chaperonin (*cpn60*) gene sequence analyses, it was shown that all the isolates were enterococci but formed

two separate lines of descent. Pairwise 16S rRNA sequence comparisons demonstrated that the two novel organisms were most closely related to each other (97.9 %) and to *Enterococcus aquimarinus* (97.8 %). Both organisms contained major amounts of C_{16:0}, C_{16:1} ω7c, and C_{18:1} ω7c/12t/9t as the major cellular fatty acids. Based on biochemical, chemotaxonomic, and phylogenetic evidence, the names *Enterococcus lemanii* sp. nov. (type strain PC32^T = CCUG 61260^T = NRRL B-59661^T) and *Enterococcus eurekensis* sp. nov. (type strain PC4B^T = CCUG 61259^T = NRRL B-59662^T) are proposed for the hitherto undescribed species.

Electronic supplementary material The online version of this article (doi:10.1007/s10482-013-9908-2) contains supplementary material, which is available to authorized users.

Author Correction to: Antonie van Leeuwenhoek (2013) 103(1): 89–98. doi:10.1007/s10482-012-9789-9.

A significant number of unfortunate errors have been identified in the above mentioned article. The full corrected article is republished on the following pages and should be treated as definitive by the reader, replacing the earlier version.

The online version of the original article can be found under doi:10.1007/s10482-012-9789-9.

M. A. Cotta · T. R. Whitehead (✉)
Bioenergy Research Unit, National Center for
Agricultural Utilization Research, USDA, Agricultural
Research Service, 1815 N. University Street, Peoria IL,
61604, USA
e-mail: Terry.Whitehead@ars.usda.gov

E. Falsen · E. Moore
Culture Collection, Department of Clinical Bacteriology,
University of Göteborg, 41346 Göteborg, Sweden

Keywords *Enterococci* · 16S rRNA · Phylogeny ·
Taxonomy · Manure · Swine

Introduction

Enterococci are Gram-positive cocci that are found in a wide range of environments that include foodstuffs, plants and water; in addition they are often considered to be commensal inhabitants of warm-blooded animals

P. A. Lawson
Department of Botany and Microbiology, University of
Oklahoma, Norman, OK 73019, USA

P. A. Lawson
Ecology and Evolutionary Biology Program, University of
Oklahoma, Norman, OK 73019, USA

but can also be found associated with reptiles and insects (Švec and Devriese 2009). Thus enterococci are commonly found in the feces of humans and other animals and their presence in water can be considered an indication of faecal pollution (Marks 2001; Miescier and Cabelli 1982). Associated with intensive modern livestock production systems are the lagoon treatment or deep pit storage methods to manage liquid swine manure. The production of odorous chemicals that include ammonia, organic acids and alcohols, and sulphides by microorganisms, and an understanding of the underlying processes of the production of these compounds is the focus of ongoing research on swine manure management (Miller 2001; Le et al. 2005; Feilberg et al. 2010). Furthermore, storage facilities such as lagoons are subject to leakages that may contaminate surrounding water sources and a better understanding of the microorganisms present including enterococcal species may provide useful public health information (Harwood et al. 2000; Marks 2001). Previous research from our laboratories on the bacterial populations present in swine feces and manure stored in underground pits has demonstrated that the vast majority of the microorganisms are low mol% G+C, Gram-positive anaerobic bacteria (Whitehead and Cotta 2001; Cotta et al. 2003). These ecosystems have also been found to be a rich source of new and novel bacterial genera and species (for example, see Whitehead et al. 2003, 2005; Cotta et al. 2009). The present study deals with our continuing investigation into the microflora of underground manure storage pits and the description of six strains of Gram-positively staining, facultatively anaerobic, coccus-shaped organisms. Comparative 16S rRNA gene sequence analyses confirmed that all the isolates were enterococci and formed two separate branches. Pairwise analysis demonstrated that the two novel organisms were most closely related to *Enterococcus aquimarinus*. In addition to phenotypic traits the distinctiveness of the two species was confirmed using the house-keeping genes *rpoA* and *cpn60*, pairwise analysis again formed a group with *E. aquimarinus*.

Based on phenotypic, chemotaxonomic, and phylogenetic considerations, it is proposed that the two unknown species originating from underground swine manure pits be classified as novel species of *Enterococcus*, for which we propose the names *Enterococcus eurekaensis* sp. nov. (type strain PC4B^T = CCUG 61259^T = NRRL B-59662^T) and *Enterococcus*

lemanii sp. nov. (type strain PC32^T = CCUG 61260^T = NRRL B-59661^T).

Methods

A number of isolates designated PC32^T (= CCUG 61260^T = NRRL B-59661^T), PPC27A (= CCUG 61369) and PPC38 (= CCUG 61261), PC4B^T (= CCUG 61259^T = NRRL B-59662^T), PPC15 (= CCUG 61368) and PPC107 (= CCUG 61272) were recovered from a swine manure storage pit located near Peoria, IL, USA. Isolations and enumerations were performed by plating samples that were serially diluted in anaerobic buffer onto habitat simulating media containing either 40 % (v/v) substrate depleted rumen fluid (Dehority and Grubb 1976; Leedle and Hespell 1980) or 80 % (v/v) clarified swine manure slurry (Slurry medium; 8,000×g, 20 min, 4 °C) (Cotta et al. 2003). The media used in these experiments were prepared anaerobically using the method of Hungate as modified by Bryant (1972). The basic media contained macrominerals, microminerals, buffers, reducing agents and other components as in the RGM medium as described by Hespell et al. (1987) or anaerobic BHI medium as described by Whitehead and Flint (1995). No additional volatile fatty acids were added to slurry containing media. Glucose, xylose, cellobiose, maltose, starch (0.05 % w/v each) and peptone (0.3 % w/v) were provided as complex carbon, nitrogen and energy sources. Plates were initially incubated anaerobically in a 96 % carbon dioxide, 4 % hydrogen atmosphere at room temperature for manure slurry samples to simulate the pit environment (Cotta et al. 2003). The new isolates were subsequently determined to grow equally well at 37 °C and cultures were routinely grown at this temperature. Single colonies were picked and repeatedly streaked out until pure cultures were obtained.

Gram-staining was performed with the BD Gram Stain Kit (Sigma, St. Louis, IL), according to manufacturer's instructions. Cell morphology and motility was examined via phase contrast microscopy using an Olympus DB12 microscope. The strains were biochemically characterised by using a combination of conventional tests, the API Rapid ID 32S API Rapid ID 32A, API 50CH and API ZYM systems, according to the manufacturer's instructions (API Biomérieux, Marcy L'Etoile, France). All biochemical tests were

performed in duplicate. Coagulase activity was determined using the Fluka Coagulase Test kit (Sigma, St. Louis, IL), according to manufacturer's instructions. *Staphylococcus aureus* was used as a positive control. Bile Esculin Agar was obtained from Difco. The presence of Lancefield A–G group antigens was assayed using the SLIDEX Strepto Plus kit (Biomérieux, Durham, NC), according to manufacturer's instructions. Other classical phenotypic tests were performed as described by Tindall et al. (2007). Hydrogen production was determined by analyses of headspace gases after 24 h of growth on BHI using a Hewlett Packard HP 5890 Gas Chromatograph equipped with a packed column of Carbosieve G (100/120 mesh, 3 mm × 2.0 mm id, Supelco) heated to 170 °C and detected by thermoconductivity with argon as the carrier (10 ml/min) and the reference gas (15 ml/min).

For the determination mol% of G+C content and PCR of genes used for phylogenetic analyses, DNA was isolated by the method of Saito and Miura (1963). To provide a rapid means of identification and determine the phylogenetic position of the swine manure isolates, 16S rRNA gene sequence analysis was performed as described by Whitehead and Cotta (2004). Sequences for *rpoA* genes were amplified as described by Naser et al. (2005). Sequences for *cpn60* were obtained as described previously by Vermette et al. (2010), and closely related sequences were obtained from the cpnDB chaperonin sequence database (<http://www.cpnDB.ca/cpnDB/home.php>). For the 16S rRNA and *rpoA* genes the closest known relatives of the new isolates were determined by performing database searches of EMBL/GenBank using the program FASTA (Pearson and Lipman 1988). These sequences and those of other related strains were aligned with the newly determined sequences using the program Clustal-W (Thompson et al. 1994) contained within the program SEQtools (Rasmussen 2002). The resulting multiple sequence alignment was corrected manually to remove the first 100 nucleotide bases to reduce alignment ambiguities using the program GeneDoc (Nicholas et al. 1997). Phylogenetic analysis was performed on 1,320 shared nucleotides phylogenetic tree was constructed according to the neighbor-joining method (Saitou and Nei 1987) with the programs SEQtools and TREEVIEW (Page 1996). The stabilities of the groupings were estimated by bootstrap analysis (1,000 replications) using the same

programs. Where necessary, pairwise sequence comparisons were determined using the same programs. For the *rpoA* gene (612 nucleotide bases) and *cpn60* (551 nucleotide bases) the same method of phylogenetic analyses were used. All major branching nodes were confirmed by maximum parsimony (data not shown). RAPD-PCR analyses were carried out with primers m13 and D11344 as previously described (Descheemaeker et al. 1997; Andrighetto et al. 1998). Determination of mol% G+C was carried out by thermal denaturation of chromosomal DNA using a Beckman model DU 640 spectrophotometer equipped with a high performance temperature controller and Tm analysis software (Johnson 1994).

Fatty acid methyl ester (FAME) analysis was performed using gas chromatography, performed in a standardised protocol using a system similar to that of the MIDI Sherlock MIS system (www.ccug.se/pages/CFA_method_2008.pdf) as described previously (Miller 1982; Sasser 1990). Cells were grown for 3 days on Chocolate agar [Brain Heart Infusion (BHI), Difco 241830] under anaerobic conditions at 37 °C. Analysis was carried out with a Hewlett Packard HP 5890 Gas Chromatograph equipped with a phenyl methyl silicone fused silica capillary column (HP-5 25 m × 0.2 mm × 0.33 mm film thickness) and a flame ionization detector. Hydrogen was used as the carrier gas. The temperature program was initiated at 170 °C and increased at 5 °C min⁻¹ to a final temperature of 270 °C. Integration of peaks and further calculations was performed by a HP 3396A integrator. FAMES were identified and quantified, and the relative amount of each fatty acid was expressed in terms of the percentage of total fatty acids in the profile of the strain.

Results and discussion

16S rRNA gene sequence analysis demonstrated that the isolates belonged to two distinct, but closely related groups. Group 1 isolates (PC32^T (= CCUG 61260^T = NRRL B-59661^T), PPC27A (= CCUG 61369), and PPC38 (= CCUG 61261) and Group 2 isolates (PC4B^T (= CCUG 61259^T = NRRL B-59662), PPC15 (= CCUG 61368) and PPC107 (= CCUG 61272). Each group consisted of strains that had a number of similar features that included being Gram-positive, non-motile coccid or ovoid-

Table 1 Characteristics useful in differentiating *E. lemanii* sp. nov., *E. eurekaensis* sp. nov., and *E. aquimarinus*

Biochemical tests given in bold typeface are the most useful

+ positive, – negative, *d* strain dependant, *w* weakly positive

^a Full profiles for API Rapid ID32S, API Rapid ID32An and API ZYM for all strains are available at <http://www.ccug.se>. Additional API test systems are also available for the Type strains at the same address

Characteristic ^a	<i>E. lemanii</i> (three strains)	<i>E. eurekaensis</i> (three strains)	<i>E. aquimarinus</i> CCUG 51308 ^T
API Rapid ID32An			
Arginine arylamidase	+	w	–
β-galactosidase	d	+	+
Leucyl glycine arylamidase	+	–	–
Leucine arylamidase	+	w	–
Pyroglutamic acid arylamidase	+	w	+
Alanine arylamidase	+	w	–
Glycine arylamidase	+	–	–
Histidine arylamidase	+	w	+
Serine arylamidase	+	w	–
API ZYM			
Esterase (C₄)	–	–	+
Esterase lipase (C ₈)	–	–	w
N-acetyl-β-glucosaminidase	+	–	+
Hydrogen from BHI growth	–	–	+
DNA G+C content (mol%)	37.9	36.0	38.7
Source	Swine manure	Swine manure	Sea water

Table 2 Cellular fatty acid compositions (%) of *E. eurekaensis* sp. nov., *E. lemanii* sp. nov., and *E. aquimarinus*

^a Data obtained from this study, all growth conditions were identical as given in the text. Bold values represent major products

Fatty acid ^a	<i>E. eurekaensis</i> sp. nov. CCUG 61259 ^T	<i>E. lemanii</i> sp. nov. CCUG 61260 ^T	<i>E. aquimarinus</i> CCUG 51308 ^T
C _{13:0}	1.8	2.7	0.6
Iso-2OH-C _{13:0}	0.9	0.6	1.5
C _{14:0}	11.2	6.6	11.6
C _{14:1} ω7c	0.3	–	–
C _{16:0}	22.2	18.0	18.1
C _{16:1} ω7c	18.9	16.1	24.0
C _{16:1} ω7c/iso-2OH-C _{15:1} ω7c/C _{16:1} ω6c	2.2	3.3	–
C _{18:0}	0.4	0.8	4.3
C _{18:1} ω9c	0.4	2.0	6.3
C _{18:2} ω6,9c/anteC _{18:0}	0.4	2.0	11.8
C _{18:1} ω7c/12t/9t	42.8	50.6	22.5

shaped cells in single cells, pairs, or short chains. All strains were catalase- and oxidase-negative.

After 48 h of anaerobic growth at 37 °C on blood agar plates, colonies varied in diameter from 0.5 to 2 mm in diameter and being whitish-grey in color and smooth and flat. No hemolysis was observed. Growth was observed at 10, 30, 37, and 45 °C. Optimal growth was observed with BHI at 37 °C. Growth was also observed in the presence of 6.5 % NaCl. Growth on BHI and Bile Esculin Agar plates under aerobic conditions at 37 °C mirrored that on blood agar. All

strains produced whitish colonies capable of hydrolysis of esculin. Such variation in growth on Bile Esculin Agar by different enterococcal species and strains has been previously demonstrated (Weiss et al. 2005). Lancefield A–G group antigens were not detected. According to the manufacturer's information such a result is not unusual with hemolysis-negative species. The most useful biochemical and enzymatic tests used to distinguish *E. lemanii*, *E. eurekaensis* and *E. aquimarinus* are given in Table 1; with the presence or absence of leucyl glycine arylamidase, glycine

arylamidase, esterase (C_4), and N -acetyl- β -glucosaminidase being the most discriminative. Cellular fatty acid analysis demonstrates that both novel organisms contain major amounts of $C_{16:0}$, $C_{16:1}$ $\omega 7c$, and $C_{18:1}$ $\omega 7c/12t/9t$ (Table 2). Using identical growth conditions PC4B^T contained almost twice as much $C_{14:0}$. Fatty acid profiles demonstrates that $C_{14:0}$, $C_{18:2}$ $\omega 6,9c/$ ante $C_{18:0}$, and $C_{18:1}$ $\omega 7c/12t/9t$ are particular useful is the differentiation between the two novel strains and *E. aquimarinus*. RAPD-PCR analyses of the strains using primers m13 and D11344 indicates that the strains are of separate clonal lineage and are distinct from *E. aquimarinus* (Supplementary Figs. 1, 2).

To investigate the phylogenetic affinity of the unknown isolates the 16S rRNA gene was amplified by PCR and sequenced. Sequence searches of EMBL/GenBank revealed that the unknown organisms were

members of the *Firmicutes* phylum and most closely related to members of the genus *Enterococcus*. A phylogenetic tree, constructed by the neighbor-joining method, depicting the phylogenetic affinity of the novel bacteria as exemplified by strain PC4B^T and PC32^T, is shown in Fig. 1. Pairwise comparisons between the two novel species (1415 nb) demonstrated that the sequences represented two distinct but closely related lines of decent within the enterococci. Each group contained sequences of very high homogeneity (99.5–100 %) and were 97.8 % related to each other. For a continuous stretch of 1500 nucleotide bases, PC32^T, PPC27A and PPC38 differed by a total of only 6 bases; likewise PC4B^T, PPC15 and PPC107 differed by only 5 bases confirming the genetic homogeneity of the two groups. The two organisms formed a cluster with *E. aquimarinus* (Švec et al. 2005) with 16S rRNA

Fig. 1 16S rRNA tree showing the phylogenetic relationships of *E. lemanii* sp. nov. and *E. eurekaensis* sp. nov., and some other *Enterococcus* species. The tree constructed using the neighbour-joining method was based on a comparison of approx. 1,320 nucleotides. *Tetragenococcus solitarius* LMG 12890^T was used as the outgroup, and bootstrap values, expressed as a percentage of 1,000 replications, are given at branching points; only significant values are shown. The bar represents a sequence divergence range of 1 %

gene sequence similarity values of 97.8 %. There is no precise correlation between 16S rRNA sequence divergence and species delineation when considering different genera, but it is generally recognised that divergence values of 3 % or more are significant (Stackebrandt and Goebel 1994). However, more recent information demonstrates that this value can be decreased to 1.3 % without loss of resolution (Stackebrandt and Ebers 2006) in that corresponding DNA–DNA hybridization values remain below 70 %, the generally accepted limit for species delineation (Wayne et al. 1987). Many enterococci share high levels of sequence similarity based on 16S rRNA gene sequence analysis and Naser et al. (2005) demonstrated the usefulness of housekeeping genes as alternative phylogenetic and identification tools. Therefore, we further investigated the distinctiveness of the unidentified strains using *rpoA* gene sequence analysis. Phylogenetic analysis employing *rpoA* as shown in Fig. 2 again showed that the novel organisms clustered with *E. aquimarinus*, strains PC32^T and PC4B^T possessing sequence similarity values of 90.9 % and

92.7 % respectively. Although Naser et al. (2005) demonstrated that interspecies variations of the *rpoA* could be based upon similarity values of <97 %, Sisteck et al. (2011) as demonstrated this value can be increased to <99 %. Similarly, sequence analysis using the universal 60 kDa chaperonin gene (*cpn60*) as been shown to offer superior discrimination between closely related organisms at the species level and as been applied to enterococci (Vermette et al. 2010). A phylogenetic tree demonstrating the phylogenetic relationships of the two novel organisms within the enterococci is shown in Fig. 3. Pairwise sequence analyses demonstrated that the novel organisms formed a cluster with *E. aquimarinus* although each displayed low sequence similarities of approximately 83 % demonstrating the separateness of these species. In all three phylogenetic analyses undertaken, the two novel species form a robust cluster with *E. aquimarinus* supported by significant bootstrap values. However, the branching node shared by *E. eurekaensis* and *E. aquimarinus* is not supported by high bootstrap values demonstrating that *E. eurekaensis* and *E. lemanii* can be

Fig. 2 *rpoA* tree showing the phylogenetic relationships of *E. lemanii* sp. nov. and *E. eurekaensis* sp. nov., and some other *Enterococcus* species. The tree constructed using the neighbour-joining method was based on a comparison of approx. 615 nucleotides. *Tetragenococcus solitarius* LMG 12890^T was used as the outgroup, and bootstrap values, expressed as a percentage of 1,000 replications, are given at branching points; only significant values are shown. The bar represents a sequence divergence range of 1 %

Fig. 3 Phylogenetic relationships of *E. lemanii* sp. nov. and *E. eurekaensis* sp. nov., and some other *Enterococcus* species based on the *cpn60* gene. The tree constructed using the neighbour-joining method was based on a comparison of approx. 605 nucleotides. *Tetragenococcus solitarius* LMG 12890^T was used as the outgroup, and bootstrap values, expressed as a percentage of 1,000 replications, are given at branching points; only significant values are shown. The bar represents a sequence divergence range of 1 %

interchangeable reflecting their identical similarity values (97.8 %) to *E. aquimarinus*.

The GenBank accession numbers for the 16S rRNA, *rpoA*, and *cpn60* gene sequences of *E. eurekaensis* strains are PC4B^T: AF445305, JQ003583, JQ038128; PPC15: AF445307, JX087947, JQ999956; PPC107: AF445284, JX087946, JQ999959. The GenBank accession numbers for the 16S rRNA, *rpoA*, and *cpn60* gene sequences of *E. lemanii* strains are: PC32^T: AF445301, JQ003584, JQ038129; PPC27A: AF445278, JQ087948, JQ999957; PPC38: AF445300, JX087949, JQ999958.

The unidentified organisms were found to be morphologically and biochemically consistent with their assignment to the genus *Enterococcus*. In particular, the organisms from swine manure form a distinct cluster with *E. aquimarinus*. In addition to their 16S rRNA, *rpoB*, and *cpn60* sequences, the two novel organisms may also be differentiated from each other and *E. aquimarinus* by characteristics shown in Table 1 and Supplementary Figs. 1 and 2. Based on phenotypic, chemotaxonomic, and phylogenetic evidence that

demonstrates the separateness of the novel organisms from other members of the genus *Enterococcus*, we consider the unidentified organisms merit classification in two new species, *Enterococcus* as *E. eurekaensis* sp. nov. (type strain PC4B^T = CCUG 61259^T = NRRL B-59662^T) and *E. lemanii* sp. nov. (type strain PC32^T = CCUG 61260^T = NRRL B-59661^T).

Description of *E. eurekaensis* sp. nov.

eurekaensis: eu. re.ken'sis N.L. masc. adj. pertaining to Eureka, a city in Illinois, USA, from where the type strain was isolated. Cells consisted of Gram-positive, catalase- and oxidase-negative, non-motile coccid or ovoid-shaped cells in single cells, pairs, or short chains. After 48 h of anaerobic growth at 37 °C on blood agar plates, colonies were 1–2 mm in diameter, grey, smooth and flat. No hemolysis was observed. Facultatively anaerobic. Grows at 45 °C but slow growth is observed at 10 °C. Growth in BHI with

6.5 % NaCl. Catalase, urease and nitrate reduction activity is negative. Hydrolyse esculin and starch but not indole or hippurate. Voges-Proskauer negative. Coagulase activity was negative. Lancefield A–G group antigens are not detected. Classical methods show that cellobiose, fructose, glucose, lactose, maltose, and sucrose are utilized but arabinose and xylose are not. For the API 50CH tests PC4B^T provided a positive reaction for esculin hydrolysis with weakly positive reactions for *N*-acetyl glucosamine, cellobiose, galactose, glucose, fructose, maltose, mannose, lactose, raffinose, salicin, sucrose, starch, trehalose, D-turanose, and D-xylose. Using the API Rapid ID32An test kit positive reactions are obtained for *N*-acetyl- β -glucosaminidase, alanine arylamidase (weak), arginine arylamidase (weak), β -galactosidase, α -glucosidase (weak), β -glucosidase, mannose, raffinose, and. Negative reactions are obtained with alkaline phosphatase, arginine dihydrolase, α -fucosidase, β -glucuronidase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, glycine arylamidase, indole hydrolysis, leucyl glycine arylamidase, nitrate reduction, proline arylamidase, and urease. Reactions for α -galactosidase, histidine arylamidase, leucine arylamidase, phenyl alanine arylamidase, pyroglutamic acid arylamidase, serine arylamidase, and tyrosine arylamidase are weak or strain dependent. α -arabinosidase and β -galactosidase 6-phosphatase and are strain dependent. Employing the API Rapid ID32S system positive reactions are produced from α -galactosidase, glycyl-tryptophan arylamidase, lactose, maltose, pyroglutamic acid arylamidase, D-raffinose, sucrose, and trehalose. Negative reactions are obtained with acetoin, β -glucosidase, β -glucuronidase, alkaline phosphatase, alanyl phenylalanine proline arylamidase, arginine dihydrolase, D-arabitol, cyclodextrin, glycogen, hippurate, β -mannosidase, melezitose, pullulan, sorbitol, tagatose, and urease. L-arabinose, *N*-acetyl- β -glucosaminidase, β -galactosidase, mannitol, melibiose, methyl β -D-glucopyranoside and D-ribose are strain dependent. Using the API ZYM system reactions for α -chymotrypsin, β -galactosidase and leucine arylamidase are positive or weakly positive depending on the strain. No activity is detected for *N*-acetyl- β -glucosaminidase, alkaline phosphatase, *N*-AS-BI-phosphohydrolase, cystine arylamidase, esterase (C4) and esterase lipase (C8), α -galactosidase, α -glucosidase, β -glucosidase, β -glucuronidase, lipase (C14), α -mannosidase, α -

fucosidase, trypsin or valine arylamidase. Acid phosphatase is strain dependent. Major fatty acids are C_{16:0}, C_{16:1} ω 7c, and C_{18:1} ω 7c/12t/9t. No hydrogen production is observed after 24 h growth on BHI. The G+C content of the DNA of the type strain is 36.0 mol%. Isolated from a swine manure storage pit. Habitat not known. The type strain is PC4B^T (= CCUG 61259^T = NRRL B-59662^T).

Description of *E. lemanii* sp. nov.

lemanii: *le.ma'ni.i* N.L. gen. mas. n. of Leman, named after the late American veterinarian Allen D. Leman for his contributions toward swine disease and swine production.

Cells consist of Gram-positive, catalase- and oxidase-negative, non-motile coccid or ovoid-shaped cells in single cells, pairs, or short chains. Growth was observed at 10, 30, 37, and 45 °C. Optimal growth was observed with BHI at 37 °C. Growth was also observed in the presence of 6.5 % NaCl. Growth on BHI and Bile Esculin Agar plates under aerobic conditions at 37 °C mirrored that on blood agar. All strains produced whitish colonies (0.5–2 mm) capable of hydrolysis of esculin. No hemolysis was observed. Facultatively anaerobic. Grows at 45 °C but slow growth is observed at 10 °C. Growth in BHI with 6.5 % NaCl. Indole production is negative. Catalase, urease and nitrate reduction activity is negative. Hydrolyse esculin and starch but not indole or hippurate. Voges-Proskauer negative. Coagulase activity was negative. Lancefield A–G group antigens were not detected. Classical methods showed that cellobiose, fructose, glucose, lactose, maltose, and sucrose were utilized but arabinose and xylose are not. Using the API 50CH test system PC32^T gave positive reactions for *N*-acetyl glucosamine, L-arabinose, cellobiose, esculin hydrolysis, galactose, glucose, fructose, mannose, salicin, maltose and D-xylose; weakly positive reactions for arabin, gluconate, lactose, melibiose, raffinose, rhamnose, sucrose, trehalose and D-turanose. Using the API Rapid ID32An, positive reactions are obtained for *N*-acetyl- β -glucosaminidase, alanine arylamidase, α -arabinosidase, arginine arylamidase, β -galactosidase 6-phosphatase, α -glucosidase, β -glucosidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, leucyl glycine arylamidase, mannose, phenyl alanine arylamidase,

pyroglutamic acid arylamidase, raffinose, serine arylamidase, and tyrosine arylamidase. Negative reactions are obtained with alkaline phosphatase, arginine dihydrolase, β -glucuronidase, glutamic acid decarboxylase, glutamyl glutamic acid arylamidase, indole hydrolysis, nitrate reduction, proline arylamidase and urease. α -fucosidase and β -galactosidase are strain dependent. Employing the API Rapid ID32S system positive reactions are produced from L-arabinose, α -galactosidase, glycyl-tryptophan arylamidase, lactose, maltose, mannitol, melibiose, methyl β -D-glucopyranoside, pyroglutamic acid arylamidase, D-raffinose, sucrose, and trehalose. Negative reactions are obtained with acetoin, β -glucosidase, alkaline phosphatase, alanyl phenylalanine proline arylamidase, arginine dihydrolase, D-arabitol, cyclodextrin, β -glucuronidase, glycogen, hippurate, melezitose, pullulan, D-ribose, sorbitol, tagatose, and urease. N-acetyl- β -glucosaminidase, β -galactosidase, and β -mannosidase were found to be strain dependent. Using the API ZYM system, positive reactions are obtained for N-acetyl- β -glucosaminidase, α -chymotrypsin, β -galactosidase, and leucine arylamidase. No activity is detected for alkaline phosphatase, N-AS-BI-phosphohydrolase, esterase (C4), esterase lipase (C8), α -glucosidase, β -glucuronidase, lipase (C14), α -mannosidase, α -fucosidase, or trypsin. Acid phosphatase, cystine arylamidase, α -galactosidase, and β -glucosidase are weakly positive or strain dependant. Valine arylamidase is strain dependent. The G+C content of the DNA of the type strain is 37.9 mol%. Major fatty acids are C_{14:0}, C_{16:0}, C_{16:1} ω 7c, and C_{18:1} ω 7c/12t/9t. No hydrogen production is observed after 24 h growth on BHI. Isolated from a swine manure storage pit. Habitat not known. The type strain is PC32^T (= CCUG 61260^T = NRRL B-61260^T).

Acknowledgments The authors wish to acknowledge Janet Hill, Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Canada for assistance with the analysis of the cpn60 gene sequences. The authors also wish to acknowledge the valuable technical assistance by Rhonda Zeltwanger.

References

- Andrighetto C, De Dea P, Lombardi A, Neviani E, Rossetti L, Giraffa G (1998) Molecular identification and cluster analysis of homofermentative thermophilic lactobacilli isolated from dairy products. *Res Microbiol* 149:631–643
- Bryant MP (1972) Interactions among intestinal microorganisms. *Am J Clin Nutr* 25:1485–1487
- Cotta MA, Whitehead TR, Zeltwanger RL (2003) Isolation, characterization and comparison of bacteria from swine faeces and manure storage pits. *Environ Microbiol* 5:737–745
- Cotta MA, Whitehead TR, Falsen E, Moore E, Lawson PA (2009) *Robinsoniella peoriensis* gen. nov., sp. nov., isolated from a swine-manure storage pit and a human clinical source. *Int J Syst Evol Microbiol* 55:150–155
- Dehority BA, Grubb JA (1976) Basal medium for the selective enumeration of rumen bacteria utilizing specific energy sources. *Appl Environ Microbiol* 32:703–710
- Descheemaeker P, Lammens C, Pot B, Vandamme P, Goossens H (1997) Evaluation of arbitrarily primed PCR analysis and pulse-field gel electrophoresis of large genomic DNA fragments for identification of enterococci important in human medicine. *Int J Syst Bacteriol* 47:555–561
- Feilberg A, Dezhao L, Adamsen A, Hansen M, Jonassen K (2010) Odorant emissions from intensive pig production measured by online proton-transfer-reaction mass spectrometry. *Environ Sci Technol* 44:5894–5900
- Harwood VJ, Whitlock J, Withington V (2000) Classification of antibiotic resistance patterns of indicator bacteria by discriminant analysis: use in predicting the source of fecal contamination in subtropical waters. *Appl Environ Microbiol* 66:3698–3704
- Hespell RB, Wolf R, Bothast RJ (1987) Fermentation of xylans by *Butyrivibrio fibrisolvens* and other ruminal bacteria. *Appl Environ Microbiol* 53:2849–2853
- Johnson JL (1994) Similarity analysis of DNAs. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) *Methods for general and molecular bacteriology*. ASM, Washington, pp 656–682
- Le P, Aarnink A, Ogink N, Becker P, Verstegen M (2005) Odour from animal production facilities: its relationship to diet. *Nutr Res Rev* 18:3–30
- Leedle JA, Hespell RB (1980) Differential carbohydrate media and anaerobic replica plating techniques in delineating carbohydrate-utilizing subgroups in rumen bacterial populations. *Appl Environ Microbiol* 39:709–719
- Marks R (2001) Cesspools of shame: how factory farm lagoons and sprayfields threaten environmental and public health. Report from NRDC and the Clean Water Network. <http://www.nrdc.org/water/pollution/cesspools/cessinx.asp>
- Miescier JJ, Cabelli VJ (1982) Enterococci and other microbial indicators in municipal wastewater effluents. *J Water Pollut Control Fed* 54:1599–1606
- Miller LT (1982) Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* 16:584–586
- Miller DN (2001) Accumulation and consumption of odorous compounds in feedlot soils under aerobic, fermentative, and anaerobic respiratory conditions. *J Anim Sci* 79:2503–2512
- Naser SM, Thompson FL, Hoste B, Gevers D, Dawyndt P, Vancanneyt M, Swings J (2005) Application of multilocus sequence analysis (MLSA) for rapid identification of *Enterococcus* species based on rpoA and pheS genes. *Microbiology* 151:2141–2150

- Nicholas KB, Nicholas HB Jr, Deerfield DW II (1997) GeneDoc: analysis and visualization of genetic variation. EMBNEW News 4:14
- Page RD (1996) TreeView: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358
- Pearson WR, Lipman DJ (1988) Improved tools for biological sequence comparison. Proc Natl Acad Sci USA 85: 2444–2448
- Rasmussen SW (2002) SEQtools, a software package for analysis of nucleotide and protein sequences. Published on the Internet at <http://www.seqtools.dk>
- Saito H, Miura K-I (1963) Preparation of transforming deoxyribonucleic acid by phenol treatment. Biochim Biophys Acta 72:619–629
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
- Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. In: MIDI Technical Note 101. MIDI, Inc., Newark
- Sistek V, Maheux AF, Boissinot M, Bernard KA, Cantin P, Cleenwerck I, de Vos P, Bergeron M. G. (2011). Two novel species, *Enterococcus ureasiticus* sp. nov. and *Enterococcus quebecensis* sp. nov., isolated from water. Int J Syst Evol Microbiol 62(Pt 6):1314–1320. doi:[10.1099/ijs.0.029033-0](https://doi.org/10.1099/ijs.0.029033-0)
- Stackebrandt E, Ebers J (2006) Taxonomic parameters revisited: tarnished gold standards. Microbiol Today 33:152–155
- Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849
- Švec P, Devriese LA (2009) Genus I. *Enterococcus*. In: de Vos P, Garrity GM, Jones D, Kreig NR, Rainey FA, Schleifer K-H, Whitman WB (eds) Bergey's manual of systematic bacteriology, 2nd edn., vol 3: The Firmicutes. Springer, New York, pp 594–607
- Švec P, Vancanneyt M, Devriese LA, Naser SM, Snauwaert C, Lefebvre K, Hoste B, Swings J (2005) *Enterococcus aquimarinus* sp. nov., isolated from sea water. Internat J Syst Evol Microbiol 55:2183–2187
- Thompson JD, Higgins DG, Gibson TJ (1994) Clustalw: improving the sensitivity for progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680
- Tindall BJ, Sikorski J, Smibert RM, Kreig NR (2007) Phenotypic characterization and the principles of comparative systematics. In: Reddy CA, Beveridge TJ, Breznak JA, Marzluf G, Schmidt TM, Snyder LR (eds) Methods for general and molecular microbiology. ASM Press, Washington, pp 330–393
- Vermette C, Russell A, Desai A, Hill J (2010) Resolution of phenotypically distinct strains of *Enterococcus* spp. in a complex microbial community using cpn60 universal target sequencing. Microb Ecol 59:14–24
- Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Triiper HG (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464
- Weiss A, Domig KJ, Kneifel W (2005) Comparison of selective media for the enumeration of probiotic enterococci from animal feed. Food Technol Biotechnol 43:147–155
- Whitehead TR, Cotta MA (2001) Characterization of microbial populations in swine feces and waste storage pits by 16S rDNA gene sequence analysis. Anaerobe 7:181–187
- Whitehead TR, Cotta MA (2004) Isolation and identification of hyper-ammonia producing bacteria from swine manure storage pits. Curr Microbiol 48:20–26
- Whitehead TR, Flint HJ (1995) Heterologous expression of an endoglucanase gene (endA) from the ruminal anaerobe *Ruminococcus flavefaciens* 17 in *Streptococcus bovis* and *Streptococcus sanguis*. FEMS Microbiol Lett 126:165–169
- Whitehead TR, Cotta MA, Collins MD, Lawson PA (2003) Description of *Hespellia stercorisuis* gen. nov., sp. nov., and *Hespellia porcinius* sp. nov., isolated from manure storage pits. Int J Syst Evol Microbiol 54:241–245
- Whitehead TR, Cotta MA, Collins MD, Falsen E, Lawson PA (2005) *Bacteroides coprosuis* sp. nov., isolated from swine-manure storage pits. Int J Syst Evol Microbiol 55:2515–2518