Systematic and Applied Microbiology 39 (2016) 160–163

Contents lists available at ScienceDirect

Systematic and Applied Microbiology

journal homepage: www.elsevier.de/syapm

Bergeyella porcorum sp. nov., isolated from pigs

L. Zamora[a], L. Domínguez[a], J.F. Fernández-Garayzábal[a,b], A.I. Vela[a,b,*]

[a] Centro de Vigilancia Sanitaria Veterinaria (VISAVET), Universidad Complutense, 28040 Madrid, Spain
[b] Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense, 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 12 November 2015
Received in revised form 14 March 2016
Accepted 18 March 2016

Keywords:
Bergeyella porcorum
Taxonomy
Phylogeny
16S rRNA
Pigs

ABSTRACT

Four Gram-stain-negative, catalase- and oxidase-positive, bacillus-shaped bacterial isolates were recovered from the lungs and tonsils of four pigs. Based on cellular morphology and biochemical criteria the isolates were tentatively assigned to the genus *Bergeyella*, although the organisms did not appear to correspond with *Bergeyella zoohelcum*, the only validly named species of this genus. 16S rRNA gene sequencing demonstrated that isolates represented a distinct subline within the genus *Bergeyella* with <97%. 16S rRNA gene sequence similarity with *B. zoohelcum* ATCC 43767^T. The predominant cellular fatty acids of strain 1350-03^T were iso-$C_{15:0}$ and iso-$C_{17:0}$ 3-OH and the major quinone was MK-6. The DNA G+C content of strain 1350-03^T was 37.7 mol%. The novel isolates can be phenotypically distinguished from *B. zoohelcum* based on physiological traits. On the basis of both phenotypic and phylogenetic findings, we describe a new species of the genus *Bergeyella* for which we propose the name of *Bergeyella porcorum* sp. nov. (1350-03^T=CCUG 67887^T=CECT 9006^T).

Introduction

The genus *Bergeyella* was proposed in 1994 by Vandamme et al. [27] to accommodate Gram-stain negative, non-spore-forming, oxidase-positive and non-fermenting bacteria formerly included in the genus *Weeksella*. The genus included a single species, *Bergeyella zoohelcum* (formerly *Weeksella zoohelcum*). Since that time the genus has remained invariable and no new species have been described so far [http://www.bacterio.net]. A new species of *Bergeyella*, *Bergeyella cardium*, has been recently described associated with clinical problems in humans [24], but was not formally described and the name, as to date, not been validated and therefore has no standing in the literature.

B. zoohelcum has been associated with rare but severe human clinical cases such as cellulitis, leg abscess and septicemia, after bites of cats or dogs, [13,14,16,17,20,22]. In animals, *B. zoohelcum* has been showed to be part of the oral and/or nasal microbiota of dogs and cats and other mammals [2,4,9,25], but it has been rarely involved in animal disease. To date only one study related this bacterium with respiratory disease in a cat [7]. In the present study, we report the phenotypic, genotypic and phylogenetic characterization of four isolates from one healthy pig and three diseased pigs presenting clinical symptoms indicative of respiratory disease. Based on the results of this polyphasic taxonomic study, a new species, *Bergeyella porcorum* is proposed.

Materials and methods

Bacterial isolation

Samples from lungs of three pigs and tonsils from a fourth pig were sent to the Centro Visavet at the Veterinary School in Madrid, Spain, for routine bacteriological analysis. Samples were cultured on Columbia agar plates (bioMérieux) and incubated at 37 °C for 24 h under aerobic and anaerobic [with 4–10% CO_2 using GasPak Plus (BBL) system] conditions. From each sample, representative colonies were selected for further analysis. Four Gram-negative, bacillus-shaped bacteria were recovered from lungs (1350-03^T, DIC11-00233-2A, DICM11-00234-2A) and tonsil (612A-03) of four different pigs.

16S rRNA gene sequencing

The phylogenetic affinity of the four isolates was established by sequencing of their 16S rRNA genes. A large continuous fragment (approximately 1400 bases) of the 16S rRNA gene of the four isolates was determined from PCR-amplified products, derived from universal primers pA (5′-AGAGTTTGATCCTGGCTCAG;

* Corresponding author at: Centro de Vigilancia Sanitaria Veterinaria (VISAVET), Universidad Complutense, 28040 Madrid, Spain. Tel.: +34 91 3943709; fax: +34 91 3943908.
E-mail address: avela@vet.ucm.es (A.I. Vela).

http://dx.doi.org/10.1016/j.syapm.2016.03.006
0723-2020/

positions 8-27, *Escherichia coli* numbering) and pH* (5′-AAGGAGGTGATCCAGCCGCA; positions 1541-1522). The PCR reaction was carried out in a volume of 100 μl, using 2.5 U *Taq* polymerase (Boehringer Mannheim), about 350 ng DNA, 500 nM of each primer and 200 μM dNTPs, in the appropriate buffer. After 2 min denaturation at 94 °C, the following cycle was repeated 30×: 1 min denaturation at 94 °C, 1.5 min annealing at 55 °C and 1.30 min polymerization at 72 °C. The last cycle was followed by 10 min elongation at 72 °C. The amplified product was sequenced bidirectionally using universal primers pA (5′-AGAGTTTGATCCTGGCTCAG; positions 8-27, *E. coli* numbering), pH* (5′-AAGGAGGTGATCCAGCCGCA; positions 1541–1522), antiKK (5′-CGTGCCAGCAGCCGCGGTAAT; positions 517-537) and 3 (5′-GTTGCGCTCGTTGCGGGACT). The identifications of the phylogenetic neighbours and calculations of pair-wise 16S rRNA gene sequence similarities were achieved using the EzTaxon, server ([10]; http://eztaxon-e.ezbiocloud.net/). Sequences of the most closely related species and other representative species within the family *Flavobacteriaceae* were retrieved from GenBank and aligned with the newly determined sequences, using the program, SeqTools [19]. Phylogenetic trees were constructed according to three different algorithms: Neighbour-Joining [21], using the programs SeqTools and TREEVIEW [18,19], Maximum-Likelihood, using the PHYML software [8]; and Maximum-Parsimony, using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 4 [12]. Genetic distances for the Neighbour-Joining and the Maximum-Likelihood algorithms were calculated by the Kimura two-parameter [11] and close-neighbour-interchange (search level = 2, random additions = 100) was applied in the Maximum-Parsimony analysis. The stabilities of the groupings were estimated by bootstrap analysis (1000 replications).

DNA G+C content

The G+C contents of the DNA of a representative isolate (strain 1350-03^T) were determined at the DSMZ (Braunschweig, Germany) by using the HPLC method of Mesbah et al. [15].

Fatty acid composition and respiratory quinone analyses

Respiratory quinones of strain 1350-03^T were extracted from 100 mg of freeze dried cell material, using the two-stage method described by Tindall [26], and further separated by thin layer chromatography on silica gel and analyzed, using HPLC, by the identification service of the DSMZ (Braunschweig, Germany).

Determination of the cell fatty acid-fatty acid methyl ester (CFA-FAME) of the type strain (1350-03^T) was carried out by the identification service of the DSMZ [DSMZ, Braunschweig, Germany; http://www.dsmz.de/services/services-microorganisms/identification/analysis-of-cellular-fatty-acids.html].

Morphological, physiological and biochemical characteristics

A number of key characteristics for phenotypic characterization were performed, using standard procedures [5,6,23], i.e., Gram-staining, production of catalase and oxidase, and hydrolysis of agar, DNA and starch. Growth in brain heart infusion broth was assessed at 25, 30, 37 and 42 °C, with 3.5, 4.5 and 6.5% added NaCl, and under anaerobic (with 4–10% CO_2) and microaerobic (with 5–15% O_2 and 5–12% CO_2) conditions, using GasPak Plus and CampyPak Plus systems (BBL), respectively. Growth was tested on MacConkey (bioMérieux) agar. The strains were further biochemically characterized, using the API 20NE API 20E, and APY ZYM systems (bioMérieux) according to the manufacturer's instructions. The strains were also analyzed with GN cards using the VITEK system (bioMérieux), following the manufacturer's instructions. The type strain of *B. zoohelcum* CCUG 12568^T was used as reference strain for the investigation of the phenotypic properties of pig isolates under the same laboratory conditions.

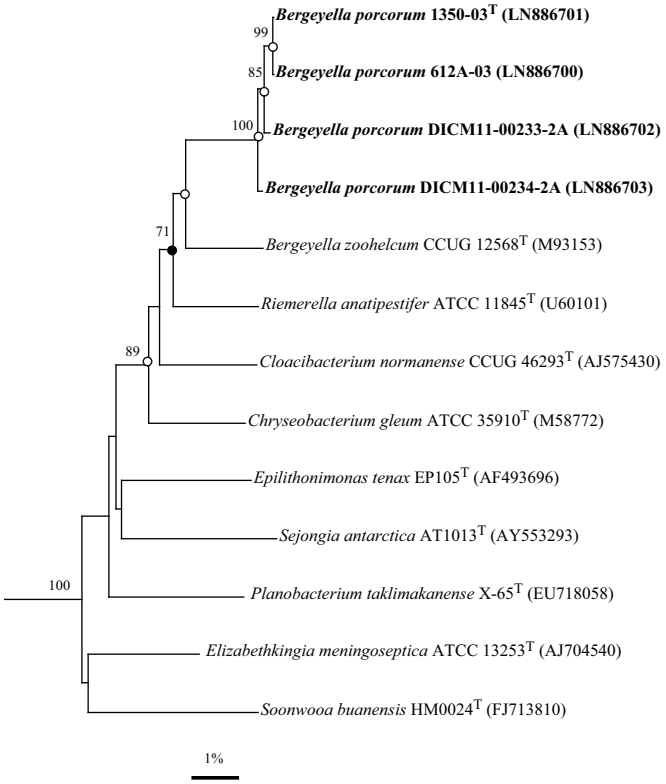

Fig. 1. Unrooted tree based on 16S rRNA showing the phylogenetic relationships of *Bergeyella porcorum* sp. nov. within the genus *Bergeyella*. *Flavobacterium aquatile* ATCC 1194^T was used as an out-group. Bootstrap values (expressed as a percentage of 1000 replications) higher than 70% are given at the branching points. Open circles indicate that the corresponding nodes (groupings) are also obtained in maximum-likelihood and parsimony trees. Filled circles indicate that the corresponding nodes (groupings) are also obtained in parsimony trees. Bar, 1% sequence divergence.

Isolate 1350-03^T has been deposited in the Spanish Type Culture collection (CECT) and in the Culture Collection of the University of Gothenburg (CCUG) Sweden, under the collection numbers CECT 9006.^{T} and CCUG 67887^T, respectively.

PFGE typing

The 4 isolates were characterized by pulsed-field gel electrophoresis (PFGE) profiling of their genomic DNA, after digestion with the restriction enzyme *Xba*I, according to the specifications of Zamora et al. [28]. Similarities between restriction endonuclease digestion profiles were based on visual comparison of the band patterns of isolates run in the same gel by the use of the BioNumerics software (version 3.0; Applied Maths, Kortrijk, Belgium) for comparisons.

Results and discussion

Comparative 16S rRNA gene sequence analysis revealed 99.5–100% sequence similarity between the isolates, thereby demonstrating their high genealogical relatedness. Sequence searches showed that the 16S rRNA gene sequence of the isolates (as exemplified by strain 1350-03^T) exhibited the highest sequence similarities with *B. zoohelcum* CCUG 12568^T (96.3% sequence similarity). The phylogenetic trees, based on the Neighbour-Joining algorithm (Fig. 1) revealed that the four isolates clustering together and formed a distinct lineage to *B. zoohelcum* CCUG 12568^T. Bootstrap resampling analysis demonstrates a strong association