

'*Arcobacter porcinus*' sp. nov., a novel *Arcobacter* species uncovered by *Arcobacter thereius*

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Abstract

Arcobacter thereius is a species associated with human disease. A group of *A. thereius* pork strains (represented by strain LMG 24487) clustered separately from the type strain (LMG 24486^T) in the 16S rRNA and multilocus phylogenetic trees. *In silico* DNA-DNA hybridization and average nucleotide identity results between their genomes (93.3 and 51.1%) confirmed '*Arcobacter porcinus*' (LMG 24487^T) as a new species. © 2016 The Author(s). Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

Keywords: ANI, *Arcobacter*, new species, pork abortion, taxonomy

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The genus *Arcobacter* belongs to the family *Campylobacteriaceae* and includes species that are considered emergent enteropathogens and potential zoonotic agents [1–4]. *Arcobacter thereius* was discovered in a study that investigated campylobacteria from pig abortions and food of animal origin [3]. This species had been later reported from faeces of patients with diarrhoea in Belgium [4] and from wastewater with an incidence of 2.1% [5]. Recently we discovered that a group of *A. thereius* strains, most isolated from pork, formed a separate cluster from the group of strains that embraced the type strain of this species (LMG 24486^T) in the 16S rRNA gene and multilocus phylogenetic (*atpA*, *gyrB*, *hsp60* and *rpoB*, 2449 bp) trees (Fig. 1 and Supplementary Fig. S1). This finding suggested that they belonged to a new species, from which we chose as a representative strain LMG 24487^T, which was recovered from liver and kidney of an aborted piglet foetus. The 16S rRNA gene sequence similarity between the latter strain (accession no. AY314754) and the type strain of *A. thereius* (LMG 24486^T,

accession no. AY314753) was 98.9%. Other species, *A. cibarius* and *A. cryaerophilus*, also show a 98.9% similarity of their 16S rRNA gene sequences [1]. Considering the availability of the genomes of the type strain of *A. thereius* LMG 24486^T and the one of strain LMG 24487^T representing the new candidate species, *in silico* DNA-DNA hybridization (isDDH) was performed, and the average nucleotide identity (ANI) between the genomes of both strains was calculated as described elsewhere [6]. The ANI and isDDH results between LMG 24486^T and LMG 24487^T were 93.3 and 51.1% (Fig. 1), both below the thresholds of 96 and 70% used for the differentiation of closely related species [6,7]. We also observed the same separation of the strains of *A. thereius* into two groups in the original description by using numerical analysis of the whole-cell protein profiles [3], and later on in another study by using amplified fragment length polymorphism analysis [8]. However, nothing was noted about the genome separation in that study.

Strain LMG 24487^T and other strains of this group are able to resist streptomycin concentrations of >48 mg/L.

The above results confirmed that strain LMG 24487^T represents a new species, for which the name '*Arcobacter porcinus*' (por.ci' nus, L. masc. adj. *porcinus* 'of pigs, pertaining to pigs') is proposed.

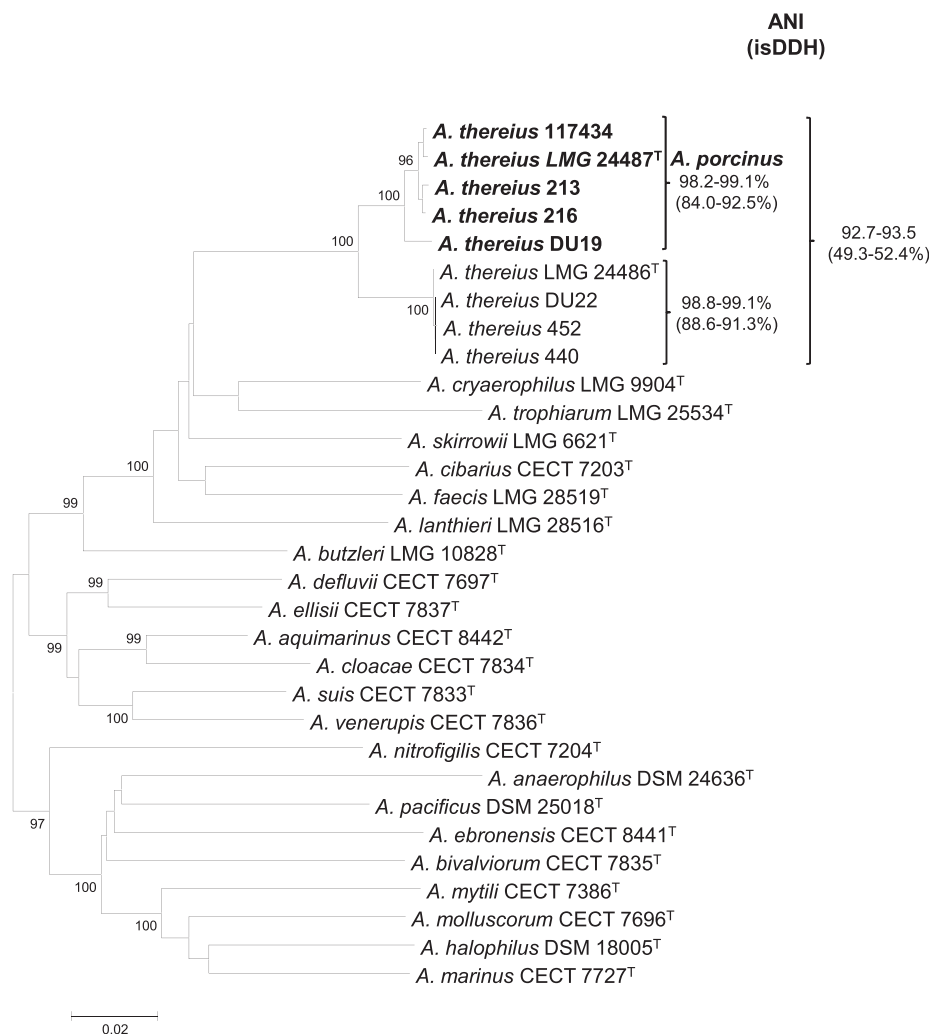


FIG. 1. Neighbour-joining phylogenetic tree obtained with concatenated sequences of four housekeeping genes (*atpA*, *gyrB*, *hsp60* and *rpoB*, 2449 bp) showing the position of the new species 'Arcobacter porcini' (bold) relative to *A. thereius* and the rest of species. Numbers at nodes represent bootstrap percentages obtained by repeating analysis 1000 times; only values higher than >95% are shown. Scale bar = 0.02 estimated substitutions per site. Average nucleotide identity values and *in silico* DNA-DNA hybridization (in brackets) represent genetic similarity obtained between genomes of two clusters.

Nucleotide sequence accession numbers

The 16S gene and genome sequences of the type strain of 'Arcobacter porcini' LMG 24487^T are available at GenBank with accession numbers AY314754 and LCUH00000000, as are the type strain of *A. thereius* LMG 24486^T, AY314753 for the 16S rRNA gene and LLKQ00000000 for the genome.

Deposit in culture collection

The type strain was already available at the Culture Collections of Belgium (LMG) and the University of Göteborg (CCUG), Sweden, with the numbers LMG 24487^T and CCUG 56899^T.

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Conflict of Interest

None declared.

Appendix A. Supplementary data

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.nmni.2016.11.014>.

References

- [1] Collado L, Figueras MJ. Taxonomy, epidemiology and clinical relevance of the genus *Arcobacter*. *Clin Microbiol Rev* 2011;24:174–92.
- [2] Figueras MJ, Levican A, Pujol I, Ballester F, Rabada Quilez MJ, Gomez-Bertomeu F. A severe case of persistent diarrhoea associated with *Arcobacter cryaerophilus* but attributed to *Campylobacter* sp. and a review of the clinical incidence of *Arcobacter* spp. *New Microb New Infect* 2014;2:31–7.
- [3] Houf K, On SLW, Coenye T, Debruyne L, De Smet S, Vandamme P, et al. *Arcobacter thereius* sp. nov., isolated from pigs and ducks. *Int J Syst Evol Microbiol* 2009;59:2599–604.
- [4] Van den Abeele A, Vogelaers D, Van Hende J, Houf K. Prevalence of *Arcobacter* species among humans, Belgium, 2008–2013. *Emerg Infect Dis* 2014;20:1731–4.
- [5] Levican A, Collado L, Figueras MJ. The use of two culturing methods in parallel reveals a high prevalence and diversity of *Arcobacter* spp. in a wastewater treatment plant. *BioMed Res Int* 2016, 8132058. <http://dx.doi.org/10.1155/2016/8132058>. 9 pages.
- [6] Auch AF, von Jan M, Klenk HP, Göker M. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. *Stand Genomic Sci* 2010;2:117–34.
- [7] Konstantinidis KT, Tiedje JM. Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci U S A* 2005;102:2567–72.
- [8] Debruyne L, Houf K, Doudah L, De Smet S, Vandamme P. Reassessment of the taxonomy of *Arcobacter cryaerophilus*. *Syst Appl Microbiol* 2010;33:7–14.