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TAXONOMIC DESCRIPTION
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Arcanobacterium wilhelmae sp. nov., isolated from the genital tract of a rhinoceros (*Rhinoceros unicornis*)

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Abstract

A taxonomic study using a polyphasic approach was performed on an unidentified *Arcanobacterium*-like Gram-stain-positive bacterium isolated from the genital tract of a rhinoceros. Comparative 16S rRNA gene sequencing showed that the bacterium belonged to the genus *Arcanobacterium* and was most closely related to the type strains of *Arcanobacterium canis* (98.8 % 16S rRNA gene sequence similarity), *Arcanobacterium phocisimile* (97.8 %), *Arcanobacterium phocae* (97.7 %), *Arcanobacterium haemolyticum* (97.4 %), *Arcanobacterium hippocoleae* (96.6 %), *Arcanobacterium pinnipediorum* (96.4 %) and *Arcarnobacterium pluranimalium* (95.4 %). DNA–DNA hybridization values between strain 647^T and *Arcanobacterium canis* DSM 25104^T were very low, 13.4 % (reciprocal 15.9 %). The genomic DNA G+C content of strain 647^T was 58.7 mol%. The presence of the major menaquinone MK-9(H_4) supported the affiliation of this strain to the genus *Arcanobacterium*. The polar lipid profile consisted of the major components diphosphatidylglycerol, phosphatidylcholine and an unidentified phosphoglycolipid. The results of physiological and biochemical testing clearly distinguished the unknown bacterium from other species of the genus *Arcanobacterium*. Based on these tests, it is proposed that the unknown bacterium should be classified as a representative of a novel species of the genus *Arcanobacterium* named *Arcanobacterium wilhelmae* sp. nov. The type strain is 647^T (=DSM 102162^T=BCCM/LMG 29418^T).

According to Yassin *et al.* [1] the genus *Arcanobacterium*, which was originally described by Collins *et al.* [2], was in need of a taxonomic revision. These authors split the genus *Arcanobacterium* into two genera with *Arcanobacterium abortisuis, Arcanobacterium bernardiae, Arcanobacterium bialowiezense, Arcanobacterium bonasi* and *Arcanobacterium pyogenes* being transferred to a new genus, *Trueperella*, and *Arcanobacterium haemolyticum, Arcanobacterium phocae, Arcanobacterium pluranimalium* and *Arcanobacterium hippocoleae* constituting the genus *Arcanobacterium* [1], with *A. haemolyticum* as the type species for the genus. More recently, the novel species *Arcanobacterium canis, Arcanobacterium phocisimile* and *Arcanobacterium pinnipediorum* were described [3–5].

During a routine microbiological diagnostic test, a bacterial strain isolated from vaginal discharge of a rhinoceros, showed unusual features. Applying a polyphasic taxonomic approach, this strain could be classified as a representative of a novel species within the genus *Arcanobacterium*.

Strain 647^T was isolated in January 2012 in high numbers (+++) together with streptococci of serological group L (+++), *Streptococcus dysgalactiae* subsp. *equisimilis* (++) and *Escherichia coli* (++) from a vaginal swab of Asian rhinoceros (*Rhinoceros unicornis*) Sani from Stuttgart Zoo Wilhelma, Germany. Sani, after five regular calvings with the last birth in 2008, did no longer display symptoms of heat. In addition, a yellow-beige muco-serous vaginal discharge was observed from October 2010 onwards. According to 16S rRNA gene sequencing results, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and Fourier transform infrared (FTIR) spectroscopy, the same isolate could also be recovered from various genital swabs of rhinoceros Sani in March 2013 (data not shown). Strain 647^T isolated in 2012 was used in

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the detailed characterization of the present study. The strain was cultured on sheep blood agar at 37 °C for 48 h under microaerobic conditions in a candle jar. The strain was characterized phenotypically by Gram-staining, oxidation reaction [6], motility test [7], haemolysis, CAMP-like haemolytic properties and biochemical properties, also with the help of the API Coryne test system (bioMérieux) and by 16S rRNA gene sequencing [8, 9].

The sequenced 16S rRNA gene fragment of strain 647^T was a continuous stretch of 1495 nt. Pairwise sequence similarities to closest related type strains were determined using EzTaxon (www.ezbiocloud.net; Kim *et al.*, 2012). Phylogenetic trees based on nearly full-length 16S rRNA gene sequences were reconstructed with ARB release 5.2 [10] using the 'All species living tree project' (LTPs; Yarza*et al.*, 2008) database release LTPs119 (July, 23 2015). Sequences not included in the database were added after alignment using the SILVA Incremental Aligner (SINA; v1.2.11; [11]). The alignment was re-checked manually and gene sequences between *Escherichia coli* positions 128 to 1379 (numbering according to Brosius *et al.* [12]) were included in the analysis. Phylogenetic trees were calculated with the maximum-parsimony method using DNAPARS v. 3.6 [13], the maximum-likelihood method using RAxML version 7.04 [14] with GTR-GAMMA and rapid bootstrap analysis, and the neighbour-joining methods using ARB Neighbour-joining and the Jukes–Cantor correction [15]. The phylogenetic trees were based on 100 resamplings (bootstrap analysis; [16]).

The 16S rRNA gene sequence-based phylogenetic trees showed the allocation of strain 647^T in the genus *Arcanobacterium* distinct from the genus *Trueperella* (Figs 1, 2). In all calculated trees, strain 647^T clustered with *A. canis* DSM 25104^T which was always supported by high bootstrap values (Fig. 1). Strain 647^T and *A. canis* DSM 25104^T shared 98.8 % 16S rRNA gene sequence similarity while the sequence similarity to type strains of other species of the genus *Arcanobacterium* was in the range of 95.4 to 97.8 %: *A. phocisimile* (97.8 %), *A. phocae* (97.7 %), *A. haemolyticum* (97.4 %), *A. hippocoleae* (96.6 %), *A. pinnipediorum* (96.4 %) and *A. pluranimalium* (95.4 %). Sequence similarities to type strains of members of the genus *Trueperella* ranged

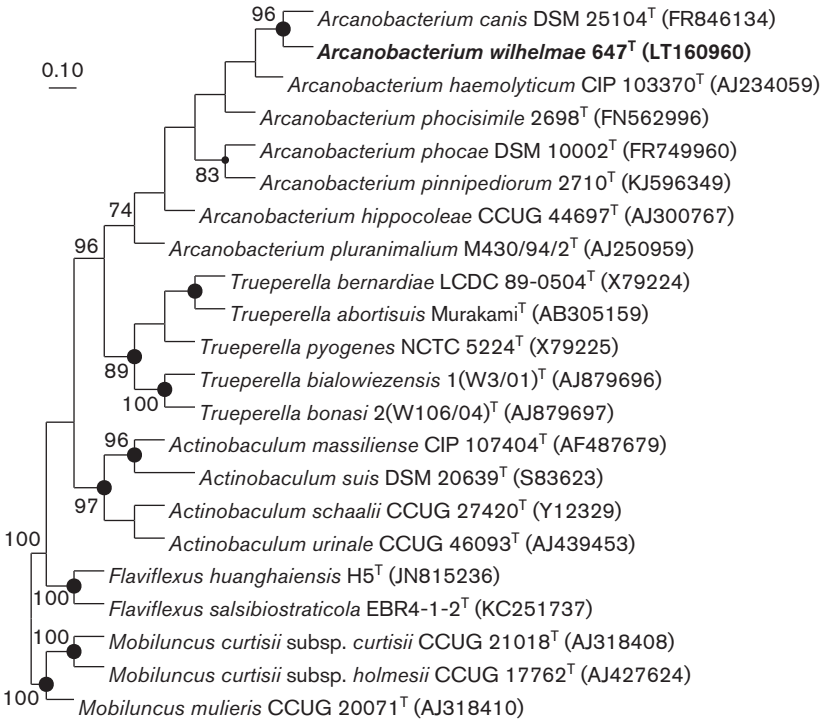

Fig. 1. Phylogenetic tree based on nearly full-length 16S rRNA gene sequences showing the position of strain 647^T within the genus *Arcanobacterium*. The tree was generated with the maximum-parsimony method using DNAPars in ARB. The tree based on DNA sequences among 16S rRNA gene termini 128 and 1379 (according to the *rrnB* gene sequence, [12]. Numbers at nodes represent bootstrap values based on 100 replications. Nodes marked with filled circles confirm phylogenetic trees generated with maximum-likelihood and neighbour-joining algorithms. Bar, 0.10 substitutions per nucleotide position.

between 94.2 and 95.0 %. Except for the pairwise sequence similarity of strain 647^T to *A. canis* DSM 25104^T, pairwise sequence similarities to other type strains were below the 98.65 % level of 16S rRNA gene sequence similarity that corresponds to the currently accepted ANI threshold for species demarcation (Kim *et al.*, 2014). In consequence, the genomic relatedness of strain 647^T and *A. canis* DSM 25104^T was investigated by DNA–DNA hybridization analysis according to the method of Ziemke *et al.* [17] with DNA extracted by the methods of Pitcher *et al.* [18]. The DNA–DNA hybridization values were very low, 13.4 % (reciprocal 15.9 %), clearly indicating that the two strains represent two different species.

The G+C content of the genomic DNA of strain 647^T and *A. canis* DSM 25104^T was determined by the DNA melting temperature method established by Gonzales and Saiz-Jimenez [19] as described previously [20] except 20 % (v/v) formamide was used in the reaction buffer to enable a better melting of the high G+C content genomic DNA. The obtained G+C content of strain 647^T was 58.7 mol% and that for *A. canis* DSM 25104^T was 51.1 mol%. The DNA G+C content of strain 647^T was slightly above that reported for species of the genus *Arcanobacterium*, while the G+C content determined for *A. canis* DSM 25104^T was in the reported range of 50–57 mol% (Yassin *et al.,* 2001). The large difference among the genomic G+C contents of strain 647^T and *A. canis* DSM 25104^T again supported the finding that the two strains represent separate species.

The results of physiological characterization are shown in Table 1 and also in the species description. In addition, the novel strain displayed no CAMP-like haemolytic reactions or a reverse CAMP reaction which are well known to be used for phenotypic identification of bacteria of the genus *Arcanobacterium* [8].

Quinones and polar lipids were extracted and analysed applying an integrated method described by Tindall [21, 22] and Altenburger *et al.* [23]. HPLC analysis was carried out as described by Stolz *et al.* [24]. The quinone system of strain 647^T was composed of 71.3 % MK-9(H_4), 17.3 % MK-8(H_4), 8.4 % MK-9 and 2.9 % MK-9(H_2), which is in agreement with quinone systems of species of the genus *Arcanobacterium* and its emended genus description [1]. The polar lipid profile (Fig. 2) contained the major lipids diphosphatidylglycerol, phosphatidylcholine) and an unidentified phosphoglycolipid (PGL4). In addition, moderate amounts of an unidentified phospholipid (PL1), phosphatidylinositol and phosphatidylinositol-mannosides and minor amounts of three unidentified phosphoglycolipids (PGL1–3) and one glycolipid (GL1) were detected. The presence of the major compounds diphosphatidylglycerol and phosphatidylcholine as well as the presence of lesser amounts of phosphatidylinositol, a phosphatidylinositol-mannoside, PGL1 and GL1 and the absence of phosphatidylglycerol was also reported for *A. canis* [3]. These shared lipids might be regarded to reflect the close phylogenetic relatedness between strain 647^T and *A. canis*. However, the

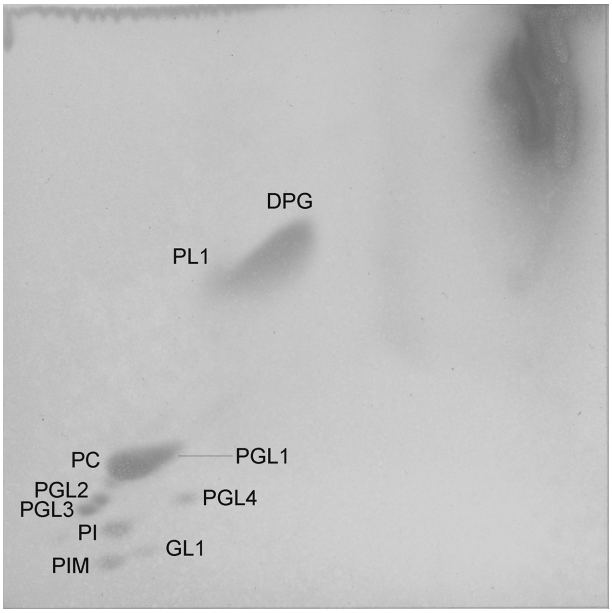

Fig. 2. Total polar lipid profile of strain 647^T after two-dimensional thin-layer chromatography and staining with molybdatophosphoric acid. DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol; PIM, phosphatidylinositol-mannoside; GL1, unidentified glycolipid; PL1, phospholipid; PGL1–4, phosphoglycolipids.

presence of the three phosphoglycolipids PGL2–4 in strain 647^T clearly distinguished the novel strain from *A. canis*.

Fatty acid analysis was performed as described previously [25]. Biomass of strain 647^T was cultured on sheep blood agar at 37 °C for 72 h and harvested at approximately the same growth stage as reported for all other species of the genus *Arcanobacterium*. Fatty acid profiling was performed in a HP 6890 gas chromatograph using the Sherlock MIDI software version 2.11 and a TSBA peak naming table version 4.1 for analysis. The fatty acid profile of strain 647^T consisted of $C_{16:0}$ (32.1 %), $C_{18:0}$ (21.8 %), $C_{14:0}$ (19.5 %), $C_{18:1}\omega 9c$ (18.5 %) and $C_{18:2}\omega 6,9c$/anteiso-$C_{18:0}$ (summed feature 5; 5.7 %) as major components, and $C_{12:0}$ (2.5 %) as a minor component. This was congruent to fatty acid profiles of other species of the genus *Arcanobacterium* [1, 3, 5]. Fatty acid profiles published by Hijazin *et al.* [3] and Sammra *et al.* [5] were analysed in the same laboratory as the data represented here by the use of biomass grown under the same conditions and harvested at the same growth stage.

Peptidoglycan analysis was fulfilled as stated by Schumann [26]. The peptidoglycan structure revealed a cross-linkage type A5α (L-Lys–L-Ala–L-Lys–D-Glu) (A11.53 according to www.peptidoglycan-types.info) and was correspondent to the description of the genus *Arcanobacterium* as rectified by Yassin *et al.* [1]. Physiological characterization was implemented as described heretofore [3, 4]; results for strain 647^T are given in the species description and are

Table 1. Phenotypic properties of strain 647^T and various other species of genus *Arcanobacterium*

Strains: 1, 647^T; 2, *A. canis* DSM 25104^T; 3, *A. phocae* DSM 10002^T; 4, *A. phocisimile* DSM 26142^T; 5, *A. pinnipediorum* DSM 28752^T; 6, *A. haemolyticum* DSM 20595^T; 7, *A. hippocoleae* DSM 15539^T; 8, *A. pluranimalium* DSM 13483^T. Data for strains 2, 3, 4, 6, 7 and 8 are mostly obtained from Sammra *et al.* [5]. All strains are positive for utilization of D-glucose as sole carbon source (API Coryne), and negative for urease activity (API Coryne), nitrate reduction (API Coryne except strain 6), serolysis on Loeffler agar, and utilization of D-mannitol as sole carbon source (API Coryne). ++, Enhanced positive; +(+), slightly enhanced positive; +, positive; (+), weakly positive; —, negative.

Phenotypic property	1	2	3	4	5	6	7	8
Haemolysis on:								
Sheep blood agar	+	+	+(+)	+	+	+	(+)	+
Rabbit blood agar	+	+(+)	+(+)	+	+	++	+	+(+)
CAMP-like reaction with:*								
Staphylococcus aureus β-haemolysin	—	(+)	—	—	—	—	+	+
Streptococcus agalactiae	—	—	+	+	+	+	—	—
Rhodococcus equi	—	+	+	+	+	+	—	+
Arcanobacterium haemolyticum	—	—	—	—	—	—	+	+
Reverse CAMP reaction	—	—	+	+	+	+	—	—
Pyrazinamidase	—†[a]	—[a]	—[a]	+[a]	+[a]	+	—[a]	+[a]
Pyrrolidonyl arylamidase (API Coryne)	—	—	—	—	+	—	—	+
Alkaline phosphatase	+[a]	+[a]	+[a]	(+)[a]	+[a]	+	+[a]	—[a]
β-Glucuronidase	+[a,b,c]	+[a,b,c]	—[a,c]	—[a,c]	—[a,c]	—[a,b,c]	+[a,c]	+[a,b,c]
β-Galactosidase	—[a,c]	+[a,c]	+[a,c]	+[a,c]	+[a,c]	+[a,c]	+[a,c]	—[a], (+)[c]
α-Glucosidase	+[a,b,c]	+[a,c]	+[a,c]	+[a,b,c]	+[a,c]	+[a,b,c]	+[a,c]	—[a,b,c]
β-Glucosidase (substrate tablets)	—	—	—	—	—	—	—	+
N-Acetyl-β-Glucosaminidase	—[a,c]	+[a,c]	+[a,c]	—[a], +[c]	+[a,c]	+[a,c]	+[a,c]	—[a,c]
Hydrolysis of aesculin (API Coryne)	—	—	—	—	—	—	(+)	+
Hydrolysis of gelatine (API Coryne)	—	—	—	—	—	—	—	+
Substrate utilization as a sole carbon source from (API Coryne):								
D-Ribose	+	+	+	+	—	+	—	+
D-Xylose	—	—	(+)	—	(+)	—	—	—
Maltose	+	+	+	+	+	+	+	(+)
Lactose	—	+	+	+	+	+	+	—
Sucrose	(+)	+	+	+	(+)	—	—	—
Glycogen	+	+	+	+	+	—	—	—
Catalase	—	—	+	+	+	—	—	+
Caseinase	—	—	—	—	—	—	—	+
Amylase	+	+	+	+	(+)	—	+	+

*Synergistic CAMP-like reaction with indicator strains.

†Results determined with: *a*, API Coryne test system (BioMérieux); *b*, tablets containing substrates (Rosco Diagnostica A/S, Taastrup, Denmark); *c*, 4 methylumbelliferyl conjugated substrates (Sigma).

correlated with the type strains of other species of the genus *Arcanobacterium* in Table 1.

Based on these taxonomic studies, strain 647^T represents a novel species of genus *Arcanobacterium*, for which the name *Arcanobacterium wilhelmae* sp. nov. is proposed.

DESCRIPTION OF *ARCANOBACTERIUM WILHELMAE* SP. NOV.

Arcanobacterium wilhelmae (wil.hel'mae. N.L. gen. n. *wilhelmae*, of the Wilhelma zoo in Stuttgart where the type strain was isolated).

Cells are Gram-stain-positive, oxidase-negative, non-motile, non-spore-forming rods (1–2 µm long and 0.5 µm wide). No CAMP-like reactions are observed with any of the indicator strains *Staphylococcus aureus*, *Streptococcus agalactiae, Rhodococcus equi and Arcanobacterium haemolyticum*. Substrate utilization as sole carbon source is obtained from D-glucose, D-ribose, maltose, D-sucrose and glycogen, but not for D-xylose, D-mannitol and lactose. Activity of the following enzymes is observed: alkaline phosphatase, β-glucuronidase and α-glucosidase. No enzyme activity is detected for the following enzymes: nitrate reductase, pyrazinamidase, pyrrolidonyl arylamidase, β-galactosidase, *N*-acetyl-β-glucosaminidase, aesculin hydrolysis, for serolysis on Loeffler agar, urease, gelatinase and catalase. Major fatty acids are $C_{16:0}$, $C_{18:0}$, $C_{18:1}\omega9c$ and $C_{14:0}$, followed by summed feature 5 ($C_{18:2}\omega6,9c$/anteiso-$C_{18:0}$); $C_{12:0}$ is present as

a minor fatty acid. The quinone system is composed of MK-9(H_4), MK-8(H_4), MK-9 and MK-9(H_2) (1 : 0.24 : 0.12 : 0.04). The polar lipid profile is composed of the major lipids diphosphatidylglycerol, phosphatidylcholine and an unidentified phosphoglycolipid (PGL4). In addition, moderate amounts of an unidentified phospholipid (PL1), phosphatidylinositol and phosphatidylinositol-mannosides and minor amounts of three unidentified phosphoglycolipids (PGL1–3) and one glycolipid (GL1) are detected. The peptidoglycan structure is of cross-linkage type A5α (L-Lys–L-Ala–L-Lys–D-Glu) (A11.53 according to www.peptidoglycan-types.info).

The type strain, 647^T (=DSM 102162^T=BCCM/LMG 29418^T), was isolated from the genital tract of a female rhinoceros. The G+C content of the genomic DNA of the type strain is 58.7 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The authors certify that the manuscript does not proclaim any work which requires approval by ethics committee(s).

References

1. Yassin AF, Hupfer H, Siering C, Schumann P. Comparative chemotaxonomic and phylogenetic studies on the genus *Arcanobacterium* Collins *et al.* 1982 emend. Lehnen *et al.* 2006: proposal for *Trueperella* gen. nov. and emended description of the genus *Arcanobacterium*. *Int J Syst Evol Microbiol* 2011;61:1265–1274.
2. Collins MD, Jones D, Schofield GM. Reclassification of *'Corynebacterium haemolyticum'* (MacLean, Liebow & Rosenberg) in the genus *Arcanobacterium* gen.nov. as *Arcanobacterium haemolyticum* nom.rev., comb.nov. *J Gen Microbiol* 1982;128:1279–1281.
3. Hijazin M, Prenger-Berninghoff E, Sammra O, Alber J, Lämmler C *et al.* *Arcanobacterium canis* sp. nov., isolated from otitis externa of a dog, and emended description of the genus *Arcanobacterium* Collins *et al.* 1983 emend. Yassin *et al.* 2011. *Int J Syst Evol Microbiol* 2012;62:2201–2205.
4. Hijazin M, Sammra O, Ulbegi-Mohyla H, Nagib S, Alber J *et al.* *Arcanobacterium phocisimile* sp. nov., isolated from harbour seals. *Int J Syst Evol Microbiol* 2013;63:2019–2024.
5. Sammra O, Balbutskaya A, Ülbegi-Mohyla H, Nagib S, Lämmler C *et al.* *Arcanobacterium pinnipediorum* sp. nov., isolated from a harbour seal. *Int J Syst Evol Microbiol* 2015;65:4539–4543.
6. Bisping W, Amtsberg G. Gram method. In: Bisping W and Amtsberg G (editors). *Colour Atlas for the Diagnosis of Bacterial Pathogens in Animals*. Berlin and Hamburg: Paul Parey Scientific Publishers; 1988. p. 336.
7. Hitchens AP. Advantages of culture mediums containing small percentages of agar. *J Infect Dis* 1921;29:390–407.
8. Ulbegi-Mohyla H, Hassan AA, Kanbar T, Alber J, Lämmler C *et al.* Synergistic and antagonistic hemolytic activities of bacteria of genus *Arcanobacterium* and CAMP-like hemolysis of *Arcanobacterium phocae* and *Arcanobacterium haemolyticum* with *Psychrobacter phenylpyruvicus*. *Res Vet Sci* 2009;87:186–188.
9. Sammra O, Balbutskaya A, Nagib S, Alber J, Lämmler C *et al.* Properties of an *Arcanobacterium haemolyticum* strain isolated from a donkey. *Berl Muench Tieraerztl Wochenschr* 2014;127:56–60.
10. Ludwig W, Strunk O, Westram R, Richter L, Meier H *et al.* ARB: a software environment for sequence data. *Nucleic Acids Res* 2004; 32:1363–1371.
11. Pruesse E, Peplies J, Glöckner FO. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;28:1823–1829.
12. Brosius J, Dull T, Sleeter D, Noller H. Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *J Mol Biol* 1978;148:107–127.
13. Felsenstein J. *PHYLIP (Phylogeny Inference Package) Version 3.6*. Distributed by the author. Seattle, USA: Department of Genome Sciences, University of Washington; 2005.
14. Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 2006;22:2688–2690.
15. Jukes TH, Cantor CR. Evolution of the protein molecules. In: Munro HN (editor). *Mammalian Protein Metabolism*. New York: Academic Press; 1969. pp. 21–132.
16. Felsenstein J. Confidence limits of phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
17. Ziemke F, Höfle MG, Lalucat J, Rosselló-Mora R. Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int J Syst Bacteriol* 1998;48:179–186.
18. Pitcher DG, Saunders NA, Owen RJ. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* 1989;8:151–156.
19. Gonzalez JM, Saiz-Jimenez C. A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* 2002;4:770–773.
20. Glaeser SP, Falsen E, Martin K, Kämpfer P. *Alicyclobacillus consociatus* sp. nov., isolated from a human clinical specimen. *Int J Syst Evol Microbiol* 2013;63:3623–3627.
21. Tindall BJ. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.
22. Tindall BJ. A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* 1990;13:128–130.
23. Altenburger P, Kämpfer P, Makristathis A, Lubitz W, Busse H-J. Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* 1996;47:39–52.
24. Stolz A, Busse HJ, Kämpfer P. *Pseudomonas knackmussii* sp. nov. *Int J Syst Evol Microbiol* 2007;57:572–576.
25. Kämpfer P, Kroppenstedt RM. Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* 1996;42:989–1005.
26. Schumann P. Peptidoglycan structure. *Methods Microbiol* 2011;38: 101–129.
27. Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W *et al.* SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* 2007;35:7188–7196.