NOTE**Characterization of a novel *Atopobium* isolate from the human vagina: description of *Atopobium vaginae* sp. nov.**

Mar Rodriguez Jovita,¹ Matthew D. Collins,² Berit Sjöden³
and Enevold Falsen³

Author for correspondence: Matthew D. Collins. Tel: +44 118 935 7000. Fax: +44 118 926 7917.
e-mail: david.collins@bbsrc.ac.uk

¹ Department of Microbiology, BBSRC Institute of Food Research, Earley Gate, Reading RG6 6BZ, UK

² Department of Food Science and Technology, University of Reading, Reading RG6 6AP, UK

³ Culture Collection, Department of Clinical Bacteriology, University of Göteborg, S-41346, Göteborg, Sweden

Phenotypic and phylogenetic studies were performed on a hitherto undescribed micro-organism isolated from the human vagina. Comparative 16S rRNA gene sequencing studies demonstrated that the unknown strain constituted a new subline within the genus *Atopobium*. The unknown bacterium was readily distinguished from other *Atopobium* species by biochemical tests and electrophoretic analysis of whole-cell proteins. Based on phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium be classified as *Atopobium vaginae* sp. nov. The type strain of *Atopobium vaginae* is CCUG 38953^T.

Keywords: *Atopobium vaginae*, phylogeny, taxonomy, 16S rRNA analysis

The genus *Atopobium* was proposed by Collins & Wallbanks (1992) to accommodate species formerly designated *Lactobacillus minutus* (Hauduroy *et al.*, 1937), *Lactobacillus rimae* (Olsen *et al.*, 1991) and *Streptococcus parvulus* (Weinberg *et al.*, 1937). Phylogenetically, *Atopobium* species form a distinct grouping which branches off within the actinomycete line of descent (Stackebrandt & Ludwig, 1994). The closest phylogenetic relative of the genus *Atopobium* is *Coriobacterium glomerans*, isolated from the intestinal tract of red soldier bugs (*Pyrrhocoris apterus*) (Haas & König, 1988). *Atopobium* species occur in human gingival crevices (*Atopobium rimae*, *Atopobium parvulum*) and may be isolated from a range of human infections [e.g. dental abscesses (*A. rimae*, *A. parvulum*), abdominal wounds, pelvic abscesses (*Atopobium minutum*)]. They consist of anaerobic, Gram-positive elliptical cocci or rod-shaped organisms which produce major amounts of lactic acid. Despite their phylogenetic association within the actinomycete branch of the Gram-positive bacteria, *Atopobium* species are not phenotypically exceptional and consequently they are sometimes confused with the low-G+C-content lactic acid group of bacteria. During the course of a study of *Lactobacillus*-like organisms from human sources, we have used 16S rRNA gene

sequencing to characterize a hitherto unknown *Atopobium* isolate from the vagina of a healthy person. Based on phylogenetic evidence and the phenotypic properties of the organism, a new species, *Atopobium vaginae* sp. nov., is described.

Strain CCUG 38953^T was isolated from the vaginal flora of a healthy person in Göteborg, Sweden. The unidentified organism was cultured on Columbia CNA agar (Difco) supplemented with 5% horse blood at 37 °C in air plus 5% CO₂. The strain was biochemically characterized by using the API ID32AN and API ZYM systems according to the manufacturer's instructions (API bioMérieux). PAGE analysis of whole-cell proteins was performed as described by Pot *et al.* (1994). For densitometric analysis, normalization and interpretation of protein patterns, the GELCOMP GCW 3.0 software package (Applied Maths, Kortrijk, Belgium) was used. The levels of similarity between pairs of traces were expressed by the Pearson product moment correlation coefficient converted to a percentage value for convenience. The G+C content (mol%) of DNA of strain CCUG 38953^T was determined as described by Garvie (1978). The 16S rRNA gene of the isolate was amplified by PCR and directly sequenced using a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relative of the new isolates was determined by performing database searches. These sequences and those of other known

Abbreviation: RDP, Ribosomal Database Project.

The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 38953^T is Y17195.

Table 1. Biochemical tests which distinguish *A. vaginae* from other *Atopobium* species

+, Positive; —, negative; v, variable.

Enzyme production	<i>A. vaginae</i>	<i>A. minutum</i>	<i>A. parvulum</i>	<i>A. rimae</i>
Acid phosphatase	+	—	+	+
Alanine arylamidase	—	—	+	—
Arginine dihydrolase	+	+	—	—
Arginine arylamidase	+	+	+	—
Histidine arylamidase	+	—	—	—
β -Galactosidase	—	—	+	—
Leucine arylamidase	+	—	+	—
Proline arylamidase	+	+	—	—
Pyroglutamic acid arylamidase	—	v	+	+
Glycine arylamidase	+	—	+	—
Serine arylamidase	+	—	—	—
Tyrosine arylamidase	—	—	+	—

related strains were retrieved from the GenBank or Ribosomal Database Project (RDP) databases and aligned with the newly determined sequence using the program PILEUP (Devereux *et al.*, 1984). The resulting multiple sequence alignment was corrected manually and a distance matrix was calculated using the programs PRETTY and DNADIST (using the Kimura-two correction parameter) (Felsenstein, 1989). A phylogenetic tree was constructed according to the neighbour-joining method with the program NEIGHBOR (Felsenstein, 1989). The stability of the groupings was estimated by bootstrap analysis (500 replications) using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

The vaginal isolate CCUG 38953^T formed small pin-head sized colonies on horse blood Columbia agar at 37 °C. Microscopically, the cells consisted of very small Gram-positive elliptical cocci (approx. 0.6–0.9 µm) that occurred singly, in pairs or short chains. The cells were non-motile. Using commercial API systems, the isolate showed arginine dihydrolase, arginine arylamidase, acid phosphatase, glycine arylamidase, histidine arylamidase, leucine arylamidase, proline arylamidase and serine arylamidase activities (Table 1). No activities for alanine arylamidase, α -arabinosidase, alkaline phosphatase, chymotrypsin, ester lipase C8, esterase C4, lipase C14, α -fucosidase, cystine arylamidase, α -galactosidase, β -galactosidase, β -galactosidase 6-phosphate, α -glucosidase, β -glucosidase, glutamic acid decarboxylase, glutamylglutamic acid arylamidase, β -glucuronidase, α -mannosidase, β -N-acetylglucosaminidase, pyroglutamic acid arylamidase, tyrosine arylamidase, trypsin or urease were detected. Phenylalanine arylamidase activity was weak or negative. The isolate did not hydrolyse gelatin or aesculin, was indole-negative and did not reduce nitrate. Based on microscopic appearance and biochemical profiling, the isolate showed some resemblance to species of the genus *Atopobium*. A DNA G + C content of 44 mol % (T_m method) for the strain

was consistent with this provisional assignment (members of the genus *Atopobium* exhibit a G + C range of approx. 36–46 mol %). Based on the analysis of whole-cell protein patterns, the unidentified isolate clustered with other members of the genus *Atopobium* (Fig. 1). The unknown bacterium, however, did not display a particularly close affinity to any of the three currently described species (viz. *A. minutum*, *A. parvulum* and *A. rimae*) thereby raising the possibility that it represents a previously unrecognized species. To determine the phylogenetic position of the unknown organism, comparative 16S rRNA gene sequence analysis was performed. The almost complete gene sequence (> 1400 nt) of the unknown coccus was determined. Sequence searches of GenBank and RDP databases revealed that the unknown bacterium was phylogenetically most closely associated with the actinomycete branch of the Gram-positive bacteria. Highest 16S rRNA sequence similarities were shown with species of the genus *Atopobium* (approx. 93.0–93.4%). The next closest relative of strain CCUG 38953^T was *C. glomerans*, exhibiting approximately 87% 16S rRNA sequence similarity. Other actinomycete taxa displayed significantly lower levels of 16S rRNA relatedness (data not shown). A tree constructed by neighbour-joining depicting the phylogenetic affinities of the unknown coccus is shown in Fig. 2 and confirmed *Atopobium* species as its nearest relatives. The unknown coccus branched off from the base of the *Atopobium* clade and was more distantly related to *C. glomerans* and other reference actinomycetes. The association of the unknown bacterium with *Atopobium* was supported in 90% of bootstrap trees.

It is clear from both phenotypic and phylogenetic evidence that the unknown vaginal isolate represents a hitherto unknown *Atopobium* species. The isolate is readily distinguished from *A. minutum*, *A. parvulum* and *A. rimae* biochemically and by PAGE analysis of whole-cell proteins. It is pertinent to note that the unknown coccus displayed a sequence divergence

Fig. 1. Similarity dendrogram based on whole-cell protein pattern of *Atopobium vaginae* sp. nov. and some related species. Levels of correlation are expressed as percentages of similarity for convenience.

Fig. 2. Unrooted tree showing the phylogenetic relationships of *Atopobium vaginae* sp. nov. and some other high-G + C-content Gram-positive bacteria. The tree constructed using the neighbour-joining method was based on a comparison of approximately 1320 nt. Bootstrap values, expressed as a percentage of 200 replications, are given at branching points. Bar, 2%.

value of approximately 7% with the aforementioned *Atopobium* species. This sequence divergence is clearly indicative of a new species and is comparable to that shown between existing species of the genus *Atopobium* (approx. 16S rRNA divergence range of 3–8%) (Collins & Wallbanks, 1992; Stackebrandt & Ludwig, 1994). Thus, based on the results of phenotypic and phylogenetic analyses, we propose the classification of the unknown bacterium in the genus *Atopobium*, as *Atopobium vaginae* sp. nov.

Description of *Atopobium vaginae* sp. nov.

Atopobium vaginae (va.gi'nae. L. n. *vagina* vagina; L. gen. n. *vaginae* of the vagina).

Cells consist of small elongated Gram-positive cocci which occur singly, in pairs or short chains. Tiny pin-head colonies are formed on blood agar at 37 °C. Facultatively anaerobic. Acid is not produced from mannose or raffinose. Arginine dihydrolase, arginine arylamidase, acid phosphatase, glycine arylamidase,

histidine arylamidase, leucine arylamidase, proline arylamidase and serine arylamidase activities are detected. Weak activity for phenylalanine arylamidase may be detected. Activities for alanine arylamidase, α -arabinosidase, cystine arylamidase, chymotrypsin, ester lipase C8, esterase C4, α -fucosidase, α -galactosidase, β -galactosidase, β -galactosidase 6-phosphate, α -glucosidase, β -glucosidase, glutamic acid decarboxylase, glutamylglutamic acid arylamidase, β -glucuronidase, α -mannosidase, β -N-acetylglucosaminidase, lipase C14, pyroglutamic acid arylamidase, tyrosine arylamidase, trypsin or urease are not detected. Indole-negative. Nitrate is not reduced. Gelatin and aesculin are not hydrolysed. The G+C content of DNA is 44 mol % (T_m). Type strain is CCUG 38953^T. Isolated from the vaginal flora of a healthy person. Habitat is unknown.

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