NOTE

***Lactobacillus psittaci* sp. nov., isolated from a hyacinth macaw (*Anodorhynchus hyacinthinus*)**

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A Gram-positive, non-spore-forming, catalase-negative, facultatively anaerobic coccibacillus to rod-shaped bacterium isolated from a parrot was characterized using phenotypic and molecular taxonomic methods. The unknown bacterium phenotypically resembled lactobacilli and comparative 16S rRNA gene sequencing demonstrated that the organism represents a distinct subline within the *Lactobacillus delbrueckii* rRNA cluster of the genus. 16S rRNA sequence divergence values of > 6% with recognized *Lactobacillus* species clearly demonstrated the phylogenetic separateness of the parrot bacterium. On the basis of phylogenetic evidence and the phenotypic distinctiveness of the unknown bacterium, a new species, *Lactobacillus psittaci* sp. nov., is proposed. The type strain of *Lactobacillus psittaci* is CCUG 42378^T (= CIP 106492^T).

Keywords: *Lactobacillus psittaci*, parrot, 16S rRNA, taxonomy, phylogeny

The genus *Lactobacillus* comprises the largest group of rod-shaped organisms within the lactic acid bacteria and over 50 species are currently recognized. These bacteria are widespread in nature and are found, for example, in milk and dairy products, other fermented foods and beverages, vegetable material and sewage. Lactobacilli are also found in association with the intestinal tracts and mucous membranes of man and many animals (Hammes *et al.*, 1992; Wibowo *et al.*, 1985). Furthermore, several *Lactobacillus* species, including *Lactobacillus acidophilus*, *Lactobacillus avarius*, *Lactobacillus fermentum*, *Lactobacillus salivarius* and *Lactobacillus reuteri*, have been recovered from birds (Kvasnikov *et al.*, 1982; Fuller, 1973; Sarra *et al.*, 1985; Fujisawa *et al.*, 1984). During an investigation of some taxonomically problematic Gram-positive, catalase-negative, rod-shaped organisms from avian sources referred to the Culture Collection of the University of Göteborg (CCUG) for identification, an unknown *Lactobacillus*-like organism recovered from a diseased parrot was subjected to a polyphasic taxonomic study. Preliminary physiological and biochemical tests indicated that the isolate was possibly related to the genus *Lactobacillus*, but it could not be assigned to any recognized species. In this

article, the characteristics of this hitherto unknown bacterium and the results of a phylogenetic analysis are reported. Based on the presented findings, a new *Lactobacillus* species, *Lactobacillus psittaci* sp. nov., is described.

Strain CCUG 42378^T was isolated following a post-mortem examination of an adult male hyacinth macaw (*Anodorhynchus hyacinthinus*) found dead at a zoological garden. Tissue samples from the right pulmonary air sacs of the lung that showed considerable inflammation were removed and transferred, under aseptic conditions, onto Columbia blood agar (Difco) supplemented with 5% horse blood and incubated at 37 °C in air plus 5% CO₂ for 2 d. Further investigations found no signs of infection by fungi or mycoplasma. None of the other parrots at the zoological garden showed any symptoms of illness. The strain was characterized biochemically by using the API rapid ID32 Strep, API CORYNE and API ZYM systems according to the manufacturer's instructions (bioMérieux). The ability to produce gas from glucose was determined using Gibson's semi-solid medium, as described by Harrigan & McCance (1976). To assess the overall phenotypic resemblance of the isolate to reference *Lactobacillus* species, a comparative analysis of whole-cell protein profiles by SDS-PAGE was performed. PAGE analysis of whole-cell proteins was performed as described by Pot *et al.* (1994). Profiles

The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 42378^T is AJ272391.

Fig. 1. Unrooted tree showing the phylogenetic relationships of *Lactobacillus psittaci* sp. nov. and some related 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 500 replications, are given at branching points. Database accession numbers are given in parentheses. UoR, University of Reading database. Bar, 1% sequence divergence.

generated were compared with a comprehensive database maintained by the CCUG. The 16S rRNA genes of the isolates were amplified by PCR and sequenced directly using a *Taq* DyeDeoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). A phylogenetic tree was constructed according to the neighbour-joining method and the stability of the groupings was estimated by bootstrap analysis (Felsenstein, 1989).

The isolate from the dead parrot was recovered directly in pure culture from a sample of inflamed pulmonary tissue and consisted of Gram-positive, non-spore-forming, coccibacilli to short rod-shaped cells. The isolate was facultatively anaerobic and catalase- and oxidase-negative. When grown on Columbia horse-blood agar, its colonies were small, entire and non-pigmented. Growth was observed at both 15 and 45 °C in MRS broth. The isolate produced gas from glucose. The organism was α -haemolytic and did not produce an odour. The isolate produced acid from D-glucose, D-raffinose and sucrose, but not from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, mannitol, melibiose, melezitose, pullulan, D-ribose, sorbitol, tagatose, trehalose or D-xylose. The isolate gave positive reactions for acid phosphatase, alanine-phenylalanine-proline arylamidase, alkaline phosphatase, cystine arylamidase, α -galactosidase, α -glucosidase, β -glucosidase, leucine arylamidase, phosphoamidase and valine arylamidase (weak reaction). *N*-Acetyl- β -glucosaminidase, arginine dihydrolase, chymotrypsin, esterase C-4, ester lipase C8, lipase C14, α -fucosidase, β -galactosidase, β -glucuronidase, glycyl-tryptophan arylamidase, α -mannosidase, β -mannosidase, methyl β -D-glucopyranosidase, pyrazinamidase, pyroglutamic acid arylamidase, pyrrolidonyl arylamidase, trypsin and urease activities were not detected. The organism hydrolysed aesculin but not gelatin or hippurate. It gave a negative Voges-Proskauer test and did not reduce nitrate to nitrite. Based on the above cultural and biochemical characteristics, the isolate from the parrot resembled the genus

Lactobacillus but did not correspond to any of the currently recognized species.

To investigate the overall phenotypic resemblance of the unknown bacterium to members of this genus, whole-cell protein profiles were determined. Protein profiling showed that the isolate was phenotypically distinct from other lactobacilli. Cluster analysis of the PAGE protein profiles showed that *Lactobacillus hamsteri* was the nearest species to the unknown isolate, joining the latter at a correlation of about 65%. Other *Lactobacillus* species were more distantly related (data not shown).

In order to ascertain the phylogenetic relationships of the unknown isolate, its 16S rRNA gene was sequenced and subjected to a comparative analysis. The almost complete gene sequence (> 1400 nt) of the isolate was determined and sequence database searches showed that the bacterium was most closely related to species of rRNA group I (*Lactobacillus delbrueckii* group; see Collins *et al.*, 1991) of the genus *Lactobacillus* (approx. 91–94% sequence similarity). The results of neighbour-joining analysis are shown in Fig. 1 and confirmed the association of the unknown parrot bacterium with rRNA group I of the genus *Lactobacillus*. The bacterium was recovered at the periphery of rRNA group I, supported by a bootstrap resampling value of 100%. All the major groupings were confirmed using the maximum-parsimony program (data not shown).

It is clear from our investigation that the *Lactobacillus*-like organism recovered from a parrot is phenotypically distinct from currently recognized members of the genus. Phylogenetically, the unknown bacterium forms a distinct subline within rRNA group I, which includes *L. delbrueckii*, the type species of *Lactobacillus*, and can therefore be considered as an authentic member of this genus. The clustering of the organism at the base of rRNA group I, together with sequence divergence values of 6% or greater, demonstrate unequivocally that it merits classification as a

novel species of the genus *Lactobacillus*. Therefore, based on phenotypic and phylogenetic evidence, it is considered that the unidentified bacterium represents a novel species, for which the name *Lactobacillus psittaci* sp. nov. is proposed.

Lactobacilli are found as commensals associated with the mucous surfaces of man and many animals. Apart from dental caries (Rogosa *et al.*, 1953), these organisms have generally been considered to be non-pathogenic. In the past, occasional reports have suggested the possible association of *Lactobacillus* species with disease (Aguirre & Collins, 1993; Sharpe *et al.*, 1973; Bourne *et al.*, 1978; Lorenz *et al.*, 1982; Dickgiesser *et al.*, 1984), and it is now evident that severe *Lactobacillus* infections occur as endocarditis in patients with valve defects and as local or disseminated infections in neutropenic patients with sepsis receiving broad-spectrum antibiotics. The patients in the latter group are usually undergoing chemotherapy for leukaemia or in an immunocompromised state due to organ transplantation or AIDS (Chomarat & Espinouse, 1991; Fruchart *et al.*, 1997; Horwitch *et al.*, 1995; Patel *et al.*, 1994; Rogasi *et al.*, 1998). Recently, a *Lactobacillus rhamnosus* probiotic has been identified as the causative agent of a human liver abscess (Rautio *et al.*, 1999). Although the presence of *Lactobacillus* species in birds is well documented, to our knowledge there are no reports of lactobacilli causing disease in these hosts. The recovery of *L. psittaci* in pure culture from an inflamed air sac is indicative that this organism is an opportunistic pathogen. The formal description of *L. psittaci* will enable diagnostic laboratories to identify this bacterium and improve information on host distribution and possible clinical significance.

Description of *Lactobacillus psittaci* sp. nov.

Lactobacillus psittaci (psit.ta'ci. L. gen. masc. n. *psittaci* of the parrot, from which the organism was first isolated).

Cells are Gram-positive, non-spore-forming, coccibacilli to rod-shaped occurring as single cells or in pairs. Facultatively anaerobic. Catalase- and oxidase-negative. After 24 h incubation on Columbia horse-blood agar, colonies are 1–2 mm in diameter, colourless and do not produce an odour. α -Haemolytic. Gas is produced from glucose (Gibson's semi-solid medium). Growth is observed on acetate agar (pH 5.4). Optimum pH range is 5.5–7.5. Grows in 2% NaCl, but not in 6% NaCl. Using commercial API systems, acid is produced from D-glucose, D-raffinose and sucrose, but not from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, mannitol, melibiose, melezitose, pullulan, D-ribose, sorbitol, tagatose, trehalose or D-xylose. Positive reactions are obtained for acid phosphatase, alanine-phenylalanine-proline arylamidase, alkaline phosphatase, cystine arylamidase, α -galactosidase, α -glucosidase, β -glucosidase, leucine arylamidase, phosphoamidase and valine arylamidase

(weak reaction). Negative reactions are observed for N-acetyl- β -glucosaminidase, arginine dihydrolase, chymotrypsin, esterase C-4, ester lipase C8, lipase C14, α -fucosidase, β -galactosidase, β -glucuronidase, glycyl-tryptophan arylamidase, α -mannosidase, β -mannosidase, methyl β -D-glucopyranosidase, pyrazinamidase, pyroglutamic acid arylamidase, pyrrolidonyl arylamidase, trypsin and urease. Aesculin is hydrolysed, but gelatin or hippurate are not. Voges-Proskauer test is negative. Nitrate is not reduced to nitrite. Isolated from the lungs of a dead hyacinth macaw (*Anodorhynchus hyacinthinus*). Habitat is not known. The type strain is CCUG 42378^T (= CIP 106492^T).

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