Streptobacillus hongkongensis sp. nov., isolated from patients with quinsy and septic arthritis, and emended descriptions of the genus Streptobacillus and Streptobacillus moniliformis

Patrick C. Y. Woo,1,2,3,4 Alan K. L. Wu,5 Chi-Ching Tsang,1 Kit-Wah Leung,1 Antonio H. Y. Ngan,1 Shirly O. T. Curreem,1 Kwok-Wai Lam,1 Jonathan H. K. Chen,1 Jasper F. W. Chan1,2,3,4 and Susanna K. P. Lau1,2,3,4

1Department of Microbiology, The University of Hong Kong, Hong Kong, PR China
2State Key Laboratory of Emerging Infectious Diseases, The University of Hong Kong, Hong Kong, PR China
3Research Centre of Infection and Immunology, The University of Hong Kong, Hong Kong, PR China
4Carol Yu Centre for Infection, The University of Hong Kong, Hong Kong, PR China
5Department of Microbiology, Pamela Youde Nethersole Eastern Hospital, Hong Kong, PR China

Two bacterial strains, HKU33T and HKU34, were isolated in Hong Kong from the pus aspirated from the right peritonsillar abscess of a patient with quinsy and the left elbow joint fluid of another patient with tophaceous gout and left elbow septic arthritis, respectively. The bacteria were Gram-stain-negative, non-motile, non-spore-forming, non-haemolytic pleomorphic bacilli. They grew best on Columbia agar with 5 % defibrinated sheep blood in an anaerobic environment or aerobic environment with 5 % CO2. They also grew on chocolate agar but not on MacConkey agar. They were catalase- and cytochrome oxidase-negative. They showed a unique profile of enzyme activities distinguishable from their closely related species. Phylogenetic analysis of the complete 16S rRNA gene, and partial groEL, gyrB and recA gene sequences showed the two isolates formed a distinct branch within the family Leptotrichiaceae, being related most closely to Streptobacillus moniliformis. Hierarchical cluster analysis of mass spectra of whole-cell protein contents showed that strains HKU33T and HKU34 were closely related to each other, but were distinct from Streptobacillus moniliformis, Sneathia sanguinegens and ‘Leptotrichia amnionii’. The DNA G+C content of strain HKU33T was 26.0 ± 2.1 mol% (mean ± SD; n = 3). DNA–DNA hybridization demonstrated ≤ 45.02 % DNA relatedness between the two isolates and Streptobacillus moniliformis CCUG 13453T. A novel species, Streptobacillus hongkongensis sp. nov., is proposed to accommodate strains HKU33T and HKU34, with HKU33T (= JCM 18691T = NCTC 13659T = DSM 26322T) designated the type strain. Emended descriptions of the genus Streptobacillus and Streptobacillus moniliformis are also given.

Streptobacillus is one of the four genera within the family Leptotrichiaceae. At the time of writing, the genus Streptobacillus contains only one species, Streptobacillus moniliformis (type species). Streptobacillus moniliformis was first isolated in 1914 from the blood of a patient who had been bitten by a rat, when the bacterium was named Streptothrix ratus (Schottmüller, 1914). In 1925, the bacteria attained its current name, Streptobacillus moniliformis, the causative agent of streptobacillary rat bite fever, characterized by fever, chills, rash, headache, vomiting, myalgia, arthritis and bacteraemia (Levaditi et al., 1925). In addition...
to rat bite fever, *Streptobacillus moniliformis* is also associated with bacteremia (Torres et al., 2003), endocarditis (Kondruweit et al., 2007), amnionitis (Faro et al., 1980), brain abscess (Dijkmans et al., 1984), cutaneous abscess (Vasseur et al., 1993), female genital tract abscess (Pins et al., 1996), splenic abscess (Chulay & Lankerani, 1976), septic arthritis (Wang & Wong, 2007), spondylodiscitis with psosas abscess (Dubois et al., 2008) and synovitis (Torres et al., 2001). Microbiologically, *Streptobacillus moniliformis* is a Gram-negative, facultatively anaerobic bacillus that grows in chains. It is found naturally in the nasopharynx (Strangeways, 1933), larynx, upper trachea (Paegle et al., 1976) and middle ears of rats (Koopman et al., 1991).

Recently, we isolated two strains of *Streptobacillus*-like bacteria from two patients. The first patient was a 38-year-old previously healthy individual with quinsy. The bacterium (strain HKU33T) was isolated from the pus aspirated from the right peritonsillar abscess. The second patient was a 64-year-old man with tophaceous gout and left elbow septic arthritis. The bacterium (strain HKU34) was isolated from the left elbow joint fluid. Both patients recovered after surgical debridement/drainage and antibiotic treatment. Complete 16S rRNA gene sequencing showed that strains HKU33T and HKU34 possessed only about 94% similarity to the most closely related species, *Streptobacillus moniliformis*. Further evaluation by biochemical testing, sequencing of other housekeeping genes and DNA–DNA hybridization studies revealed that these two isolates represent a novel species of the genus *Streptobacillus*.

Strains HKU33T and HKU34 were isolated and grown on Columbia agar with 5% defibrinated sheep blood (bioMérieux) under aerobic conditions with 5% CO2. Reference strains *Streptobacillus moniliformis* CCUG 13453T, *Sneathia sanguinegens* CCUG 41628T and *Leptotrichia amnionii* CCUG 52976 were obtained from the Culture Collection, University of Göteborg, Sweden, while reference strains *Escherichia coli* ATCC 8739 and ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were obtained from the American Type Culture Collection. The two novel isolates were phenotypically characterized in detail. They were Gram-stained and assessed for the presence of catalase and cytochrome oxidase activities and the presence of haemolysis. Biochemical data were obtained using Vitek NHI (bioMérieux), Vitek GN+ (bioMérieux) and Phoenix NID (BD Diagnostic Systems) systems. Cells of strains HKU33T and HKU34 were pleomorphic (with cocobacillary, bacillary and filamentous forms), non-sporulating, Gram-stain-negative bacilli (Fig. S1a, available in the online Supplementary Material). They were non-motile and did not produce catalase or cytochrome oxidase. Identification using the Vitek system (GN+) was unsuccessful as the growth control yielded negative results. The Vitek NHI system identified both isolates as representatives of *Kingella kingae* (92% confidence) and Phoenix NID identified both isolates as representatives of *Kingella denitrificans* (90% confidence). Strains HKU33T, HKU34 and the closely related reference strains were streaked on Columbia agar with 5% defibrinated sheep blood, chocolate agar and MacConkey agar and incubated under aerobic conditions, anaerobic conditions with 5% CO2 and anaerobic conditions, achieved using the Concept 1000 anaerobic workstation (Ruskin), to test for their growth under different conditions, and this showed that strains HKU33T and HKU34 grew the best on Columbia agar with 5% defibrinated sheep blood as non-haemolytic colonies of 0.5 mm diameter after 48 h of incubation at 37 °C in an anaerobic environment or aerobic environment with 5% CO2. Only strain HKU34 grew in an aerobic environment. They could also grow on chocolate agar but did not grow on MacConkey agar (Table 1). Enzyme activity tests using the API ZYM system (bioMérieux) indicated that the two strains possessed the same unique enzyme activity profile (Table 2).

Scanning electron microscopy was performed according to our previous publications (Woo et al., 2002, 2003b, 2005). Bacterial cells of strains HKU33T and HKU34 were aflagellated pleomorphic bacilli, consistent with the Gram smear appearance. The surfaces of the bacterial cells were very rough even after several washing steps (Fig. S1b).

Complete 16S rRNA gene sequencing was performed according to our previous publications (Lau et al., 2007, 2013; Woo et al., 2003a), with slight modifications where the end regions of the gene were amplified and sequenced using the primer pairs LPW26378/LPW26129 and LPW26128/LPW26379 (Table S1), which were designed by selecting the conserved regions flanking the 16S rRNA gene from the DNA sequences of species of the family *Leptotrichiaceae* closely related to strains HKU33T and HKU34. The sequences of the PCR products were compared with sequences of closely related species in the GenBank database by multiple sequence alignment using MUSCLE 3.8 (Edgar, 2004) and the aligned sequences were trimmed using BioEdit 7.2.0 (Hall, 1999). Tests for substitution model and phylogenetic tree reconstruction, by the neighbour-joining method and maximum-likelihood method, were performed using MEGA 5.0.4 (Tamura et al., 2011). Comparative complete 16S rRNA gene sequence analysis was performed using BioEdit 7.2.0 and it revealed 99.7% sequence similarity between strains HKU33T and HKU34 while the complete 16S rRNA gene sequence of strains HKU33T showed 94.2, 91.2 and 91.1% similarity to *Streptobacillus moniliformis* CCUG 13453T, *Sneathia sanguinegens* CCUG 41628T and *L. amnionii* CCUG 52976, respectively (Fig. 1).

To further ascertain the phylogenetic position of the novel isolates, partial groEL, gyrB and recA genes of the two isolates and the reference strains were sequenced using the primers listed in Table S1 (Woo et al., 2010). Primers LPW18598 and LPW18646–LPW18648 were designed by selecting the conserved regions of the respective gene targets from the alignments of the respective DNA sequences of species of the family *Leptotrichiaceae* closely related to strains HKU33T and HKU34. Comparative partial groEL, gyrB and recA gene sequence analysis revealed 99.2, 98.6 and 98.5% similarity between strains HKU33T and HKU34, respectively.
Table 1. Profiles of growth at different conditions for strains HKU33\textsuperscript{T} and HKU34, *Streptobacillus moniliformis* CCUG 13453\textsuperscript{T}, *Sneathia sanguinegens* CCUG 41628\textsuperscript{T} and ‘*L. amnionii*’ CCUG 52976

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aerobic with 5% CO\textsubscript{2}</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aerobic</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Strains: 1, HKU33\textsuperscript{T}; 2, HKU34; 3, *Streptobacillus moniliformis* CCUG 13453\textsuperscript{T}; 4, *Sneathia sanguinegens* CCUG 41628\textsuperscript{T}; 5, ‘*L. amnionii*’ CCUG 52976. The number of ‘+’ symbols indicates the level of growth observed; –, no growth was observed.

Table 2. API ZYM profiles of strains HKU33\textsuperscript{T}, HKU34, *Streptobacillus moniliformis* CCUG 13453\textsuperscript{T}, *Sneathia sanguinegens* CCUG 41628\textsuperscript{T} and ‘*L. amnionii*’ CCUG 52976

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphatase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Esterase lipase (C8)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lipase (C14)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Leucine arylamidase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Trypsin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>x-Chymotrypsin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Naphthol-AS-Bi-phosphohydrolase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>x-Galactosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>x-Mannosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>x-Fucosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Variable results were obtained for different strains of *Sneathia sanguinegens* (Collins et al., 2001).
Fig. 1. Phylogenetic tree showing the relationship between strains HKU33T and HKU34 and closely related species, inferred from partial 16S rRNA gene sequence data (1261 nt positions of the trimmed sequence alignments) by the maximum-likelihood method using the model GTR+I+G. Bar, 0.05 substitutions per base. Numbers at nodes indicate levels of bootstrap support calculated from 1000 trees and are expressed as percentages. All names and accession numbers are given as cited in the GenBank database. Sequences that were obtained in this study are marked with an asterisk. Only nodes that were well supported by the maximum-likelihood method (≥70% bootstrap support) have their bootstrap values shown, and all of these nodes were also well supported by the neighbour-joining method (≥70% bootstrap support).

100% of the maximal achievable signal and values obtained with the other strains were compared with this standard (Table S3). Strains HKU33T and HKU34 shared 99.9% DNA–DNA relatedness, supporting that these two isolates belong to the same species while both strains HKU33T and HKU34 possessed DNA–DNA relatedness of only about 40% with Streptobacillus moniliformis CCUG 13453T, suggesting that they belong to a species distinct from Streptobacillus moniliformis. The DNA G+C content of strain HKU33T was 26.0 ± 2.1 mol% (mean ± SD; n=3) as determined by the thermal denaturation method (Marmur & Doty, 1962) with slight modifications as described in our previous publications (Lau et al., 2013; Woo et al., 2005), a value similar to that of Streptobacillus moniliformis CCUG 13453T (26.3%) (Nolan et al., 2009) and those of other members of the family Leptotrichiaceae (22–33%) (Collins et al., 2001; Harman-Smith et al., 2010; Ivanova et al., 2009).

In summary, isolates HKU33T and HKU34 from human clinical samples formed a phylogenetic lineage clustered with Streptobacillus moniliformis and distinct from other members of the family Leptotrichiaceae (Figs 1 and S2). This was also evidenced by the hierarchical cluster analysis of mass spectra of whole-cell protein contents of strains HKU33T, HKU34 and their closely related species (Fig. S3). Phenotypically, the ability of strains HKU33T and HKU34 to grow well in aerobic environment with 5% CO₂ (Table 1), their inability to produce α-glucosidase or β-glucuronidase (Table 2), and their susceptibilities to levoﬂoxacin and metronidazole (Table S2) also supported the placement of the two isolates in the genus Streptobacillus and distinguished them from Sneathia sanguinegens and ‘L. amnionii’. Moreover, phylogenetic analyses (Figs 1 and S2) and DNA–DNA hybridization (Table S3) showed that strains HKU33T and HKU34 should be recognized as a novel species different from Streptobacillus moniliformis, from which they could be phenotypically distinguished by the ability of strains HKU33T and HKU34 to produce acid phosphatase and alkaline phosphatase and their inability to produce α-chymotrypsin and leucine arylamidase (Table 2), as well as their resistance to ciproﬂoxacin (Table S2). Therefore, we conclude that strains HKU33T and HKU34 represent a novel species of the genus Streptobacillus, for which the name, Streptobacillus hongkongensis sp. nov. is proposed, with HKU33T designated the type strain. Further studies on the pathogenic potential, natural reservoir, route of transmission and clinical disease spectrum of this species are warranted.

Emended description of the genus Streptobacillus Levaditi et al. 1925

The description is emended from that given by Staley & Whitman (2010). Rods with rounded or pointed ends, or pleomorphic bacilli with coccobacillary, bacilliform and filamentous forms. Occur singly or in chains. Gram-stain-negative. Non-motile. Non-spore-forming. Non-haemolytic. Capable of growing anaerobically, aerobically...
or aerobically with 5 % CO$_2$. Capable of growing on blood agar and chocolate agar but not on MacConkey agar, or require serum or ascetic fluid for growth. Optimum temperature for growth is 35–37 °C. Positive for esterase (C4), esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase. Negative for catalase and cytochrome oxidase. Resistant to trimethoprim-sulphamethoxazole (>32 μg ml$^{-1}$). The DNA G+C content is 24–26 mol%.

The type species is *Streptobacillus moniliformis* Levaditi et al. 1925.

**Description of *Streptobacillus hongkongensis* sp. nov.**

*Streptobacillus hongkongensis* (hong.kong.en’sis. N.L. masc. adj. *hongkongensis* of or belonging to Hong Kong, the place where the type strain was isolated).

Pleomorphic bacilli, with cocobacillary, bacillary and filamentous forms. Gram-stain-negative. Non-motile. Non-sporing. Non-toxic. Grows best on Columbia agar with 5 % defibrinated sheep blood as small (0.5 mm) colonies after 48 h of incubation at 37 °C in an anaerobic environment or aerobic environment with 5 % CO$_2$. Able to grow on chocolate agar but not on MacConkey agar. Positive for acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase but negative for N-acetyl-β-glucosaminidase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucuronidase, leucine arylamidase, lipase (C14), α-mannosidase, trypsin and valine arylamidase. Negative for catalase and cytochrome oxidase. Resistant to (μg ml$^{-1}$) trimethoprim-sulphamethoxazole (>32) but sensitive to penicillin (0.008), ciprofloxacin (0.094), ceftriaxone (0.016), cefotaxime (0.023), imipenem (0.032), amoxicillin-clavulanic acid (0.047), levofloxacin (0.19), vancomycin (0.25), chloramphenicol (3) and metronidazole (4).

The type strain is CCUG 13453$^T$ (=9901$^T$ = ATCC 14647$^T$ = CCUG 2469$^T$ = DSM 12112$^T$ = NCTC 10651$^T$).

**Acknowledgements**

This work was partly supported by the Strategic Research Theme Fund, The University of Hong Kong; Committee for Research and Conference Grant, The University of Hong Kong; and a donation from Ms Eunice Lam.

**References**


