Abstract A thin, filamentous, non-motile, aerotolerant, anaerobic, gram-negative bacterium was isolated from the blood of a 46-year-old man who was diagnosed as having acute myeloid leukemia. The organism had a positive catalase reaction but was negative in indole and oxidase tests. A commercially available system failed to identify the bacterium, but 16S rRNA gene sequencing showed it to be most closely related (97% similarity) to a recently isolated *Leptotrichia* sp. The DNA base composition was 29.7% mol G+C, and the organism produced lactate as the sole end-product of glucose fermentation. These data indicate the isolate is a new species of *Leptotrichia* for which the name *Leptotrichia trevisanii* sp. nov. is proposed.

**Introduction**

*Leptotrichia buccalis* is the only species recognized in its genus. It is a catalase-negative, anaerobic, filamentous bacterium that stains gram-negative with gram-positive granules. Its ability to produce lactic acid as the only major acid from glucose fermentation distinguishes it from other closely related genera, such as *Fusobacterium*. However, the taxonomic position of some of the strains within this species is uncertain, since they are diverse and exhibit a number of differences in DNA base composition, enzyme pattern and aerotolerance [1, 2].

*Leptotrichia* strains have been implicated in cases of periodontal disease, but they have only rarely been associated with serious systemic disease, usually in immunocompromised patients [3, 4, 5, 6]. *Leptotrichia buccalis* is part of the normal oral flora of humans; it is also found in animals such as dogs and guinea pigs that are fed with commercial pellets [6]. We describe here a case of bacteremia due to a new species of *Leptotrichia* that occurred in a man with acute myeloid leukemia.

**Patient and Methods**

**Case Report**

A 46-year-old man attended a local medical clinic for treatment of a sore mouth. The gingiva were found to be inflamed, and a diagnosis of gingivitis was made. He was treated with erythromycin and topical amphotericin. Two days later he presented to the emergency department of a public hospital with sores in his mouth and a 2-day history of sore throat, fever and myalgia. Blood cultures were taken, and he was treated withceftriaxone, gentamicin and metronidazole. The sepsis resolved, and there was no recurrence of bacteremia. Laboratory investigations revealed a haemoglobin level of 100 g/l, leukocyte count of 135 $\times 10^9$ /l and platelet count of 21 $\times 10^9$ /liter. The blood film revealed a marked leukocytosis with numerous heterogeneous blasts, suggestive of an undifferentiated acute myeloid leukemia. The clotting profile was normal. Bone marrow investigations showed acute myeloid leukemia, sub-classification M1.

The patient underwent a course of induction and consolidation chemotherapy, which brought the disease into remission. The only complication was thrombosis of an internal jugular vein, which required anticoagulation therapy.

**Isolation and Phenotypic Characterization**

Only one set of anaerobic and aerobic blood culture bottles (BacT/Alert Microbial Detection System; Organon Technika, Belgium) was used for inoculation of the patient's blood taken on admission. The anaerobic bottle showed growth at 16 h, and the contents were subcultured onto several sets of plates, each of which consisted of a 6% sheep blood agar plate and a chocolate agar plate. The plates inoculated with contents from the anaerobic bottle were incubated in 5% CO$_2$ and under anaerobic conditions using the Oxoid Anaerogen system (Oxoid, UK). The plates subcultured from the aerobic bottle were incubated in an atmosphere of
5% CO₂ in air. The cultures were examined daily for growth for 7 days.

Identification of the isolate was initially attempted using the Rapid ANA II system (Innovative Diagnostic Systems, USA) and Oxoid Anident discs (Oxoid). Other phenotypic characteristics, such as growth at different temperatures and under different conditions, motility and Gram stain morphology, were performed using conventional methods [7]. The catalase test (method 2 of reference 7) was performed, taking growth from a plate and mixing it with hydrogen peroxide on a glass slide. Fermentation end-products were analysed using high-pressure liquid chromatography with 10 mM H₂SO₄ and separating the compounds on a HPX-87H ion-exclusion column (300 mm×7.8 mm; BioRad, USA). The eluted compounds were detected using a R 401 differential refractometer (Waters, USA) after growth was achieved in strictly anaerobic liquid medium containing 4 mM glucose and 1 g of yeast extract per liter [8]. The isolate was deposited with the American Type Culture Collection as ATCC 700907.

Antibiotic Susceptibility Testing

The isolate was tested for susceptibility to penicillin, metronidazole, tetracycline, erythromycin and clindamycin using the E-test (AB Biodisk, Sweden) with Mueller-Hinton agar supplemented with 5% sheep blood. As recommended by the National Committee for Clinical Laboratory Standards in their guidelines for susceptibility testing of anaerobes [9], a bacterial suspension with a turbidity equivalent to that of a number 1 McFarland standard was used, and the MICs were read after 48 h of incubation under anaerobic conditions.

Chromosomal DNA Extraction, DNA Base Composition, Polymerase Chain Reaction (PCR) and 16S rRNA Gene Sequencing

Chromosomal DNA was extracted and purified from the culture plate using a standard cetyl trimethylammonium bromide extraction method [10], as described previously [11]. The mol% G+C ratio of genomic DNA was determined using high-pressure liquid chromatography [8]. Amplification of the 16S rRNA gene by PCR, direct sequencing of the product using general oligonucleotide primers and phylogenetic analyses were performed as described previously [11]. Briefly, the sequence was aligned (using ClustalW) with those of close relatives extracted from the GenBank database and the Ribosomal Database Project II database. Other known rRNA gene sequences in the Genbank database Collection as A TCC 14201 T ; 96% sequence identity, 54 nucleotide differences). The most similar sequence (97% sequence identity, 40 nucleotide differences) was from a recently described, novel strain of Leptotrichia isolated from a neutropenic patient [12]. As

Results

The Gram stain of the positive blood culture showed long, filamentous, gram-negative bacilli. Since fusiform anaerobic bacteria were suspected, the anaerobic plate was left undisturbed for 3 days. Bacterial growth was detected on the anaerobic plate subcultured from the anaerobic bottle after 16 h of incubation at 37°C. The same organism also grew on plates incubated aerobically with 5% CO₂, although growth was always faster on anaerobic culture. Gram stain of the anaerobic growth showed a fusiform, gram-negative bacillus (0.9 µm × 10–15 µm) with tapered ends (Fig. 1). The isolate was nonmotile and sensitive to metronidazole (disc diffusion method), but it could not be identified using a Rapid ANA II panel (profile number 064160; Innovative Diagnostic Systems). The organism did not react in many of the bio-

chemical tests of the commercial system. Other biochemical tests revealed the organism to be catalase positive, indole negative and oxidase negative.

Drug sensitivity testing with the E-test method (using Mueller-Hinton agar supplemented with 5% sheep blood agar) showed the organism to be susceptible to penicillin (MIC, 0.016 µg/ml), metronidazole (MIC, 0.5 µg/ml) and tetracycline (MIC, 0.5 µg/ml), intermediately resistant to clindamycin (MIC, 6 µg/ml) and resistant to erythromycin (MIC, 256 µg/ml). Agar disc diffusion tests showed that the organism was also sensitive to chloramphenicol, imipenem, amoxicillin/clavulananate, ticarcillin/clavulanate and kanamycin, but resistant to gentamicin. High-pressure liquid chromatography analysis of the volatile and nonvolatile organic end-products showed that lactic acid was the only major product from glucose fermentation.

The mol% G+C ratio of genomic DNA of the clinical isolate was found to be 29.7% (standard deviation, 0.16; n=5). Using broad-range primers specific for bacterial 16S rRNA genes and direct sequencing of the purified PCR products, a gene sequence of 1,456 nucleotides was obtained (GenBank accession number, AF206305). When the sequence of our isolate was compared with other known rRNA gene sequences in the Genbank database and the Ribosomal Database Project II database (Michigan State University, USA), it was found to be most similar to members of the genus Leptotrichia (e.g. Leptotrichia buccalis, ATCC 14201 T ; 96% sequence identity, 54 nucleotide differences). The most similar sequence (97% sequence identity, 40 nucleotide differences) was from a recently described, novel strain of Leptotrichia isolated from a neutropenic patient [12]. As