

Bacteremic Infection with *Pantoea ananatis*

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A 73-year-old man was hospitalized for dyspnea and bilateral ankle edema. During his hospital stay he presented anal hemorrhage and developed a high fever after colonoscopy. A set of aerobic and anaerobic blood culture bottles yielded a pure culture of gram-negative rods, susceptible to all antibiotics tested. The API20E code was 1005133, resulting in a very good identification as *Pantoea* sp. Subsequent sequencing of the 16S rRNA gene revealed a final identification as *Pantoea ananatis*. The patient was given intravenous and oral therapy with piperacillin-tazobactam and ofloxacin and recovered completely from his infection.

CASE REPORT

A 73-year-old diabetes mellitus type II patient, with previous cerebrovascular accidents and deep venous thrombosis, who had been hospitalized several times for vascular problems during the past 5 years, was hospitalized for dyspnea and bilateral ankle edema but without signs of fever or chills. During his hospital stay, a colonoscopy was performed for anal hemorrhage, after which the patient developed high fever and chills. There was only a slight elevation of C-reactive protein and no leukocytosis.

The first set (one aerobic and one anaerobic bottle [BacT/Alert FA and SN; BioMérieux, Marcy l'Etoile, France]) of two blood cultures, taken immediately after the onset of fever, was positive within 12 h of incubation for gram-negative rods. Immediately after the first blood culture became positive, the patient was given intravenous therapy with piperacillin-tazobactam (two doses of 2 g each) and ofloxacin (two doses of 400 mg each), followed by 200 mg of ofloxacin twice daily for several weeks. The patient recovered completely from his infection.

Subculturing of the positive blood cultures on tryptic soy agar with 5% sheep blood (Becton Dickinson, Erembodegem, Belgium) and on McConkey agar (Becton Dickinson) yielded a pure culture of a lactose-negative, gram-negative rod, positive for β -galactosidase but negative for ornithine, arginine dehydrolase, lysine decarboxylase, urease, and adonitol. Identification with API 20E (Biomérieux) resulted in code 1005133, with a very good identification as *Pantoea* sp.

Genotypic identification was obtained by 16S ribosomal DNA (rDNA) sequence determination and tRNA-PCR. DNA extraction was carried out by using an alkaline lysis method, i.e., by suspending one colony in 20 μ l of 0.25% sodium dodecyl sulfate–0.05 N NaOH, heating at 95°C for 15 min, and carrying out a final dilution with 180 μ l of distilled water. The

complete 16S rRNA sequence was determined by amplification of the 16S rRNA gene with the primers 5'-AGTTTGAT CCTGGCTCAG and 5'-TACCTTGTTACGACTTCGTCC CA (10), and sequencing was performed as described previously (10). Comparison of the obtained 16S rDNA sequence with all known sequences in GenBank, using the BLAST software (National Center for Biotechnology Information [http://www.ncbi.nlm.nih.gov/BLAST/]), revealed a highest match with *Pantoea ananatis* with a similarity of more than 99%. Because of the sometimes high interspecies similarity values in the family of the *Enterobacteriaceae*, a dendrogram of 16S rRNA gene sequences was constructed by using the GeneBase software package (Applied Maths, De Pinte, Belgium) (Fig. 1). Pairwise alignment by using the unweighted pair group method with arithmetic mean was carried out (gap penalty of 100% and unit gap cost of 20%), followed by global alignment with *Aeromonas hydrophila* as the outgroup. Based on this, a similarity matrix was constructed, which was used for the dendrogram, using the neighbor-joining method (6).

tRNA-PCR was carried out with the outwardly directed tRNA-gene consensus primers T5A (5'-AGTCCGGTGCTC TAACCAACTGAG) and T3B (5'-AGGTCGCGGGTTCGA ATCC), thus amplifying the intergenic tRNA spacers, as described previously (1, 2, 9, 12). Electropherograms were normalized by using GeneScan Analysis software, version 2.1 (Applied Biosystems). Transformation of GeneScan tables (ABI310, MacIntosh) to tables on IBM, separation into separate digital fingerprints, and comparison of the digital tRNA fingerprints with a library of tRNA fingerprints obtained from a large collection of reference strains was done by using software described previously (2). Using tRNA-PCR and capillary electrophoresis, we obtained a DNA fingerprint composed of tRNA spacers with fragment lengths of 66.9, 73.2, 85.7, 106.0, 111.9, 119.9, 123.2, 162.8, and 196.1 bp. This was a unique fingerprint, for which no match was found in our tRNA-PCR library, since no *P. ananatis* fingerprint was present at that time. The fingerprint differed clearly from the tRNA-PCR fingerprints of the strains of *Pantoea agglomerans* (formerly *Enterobacter agglomerans*) (67.4, 85.5, 105.6, 110.4, 134.2,

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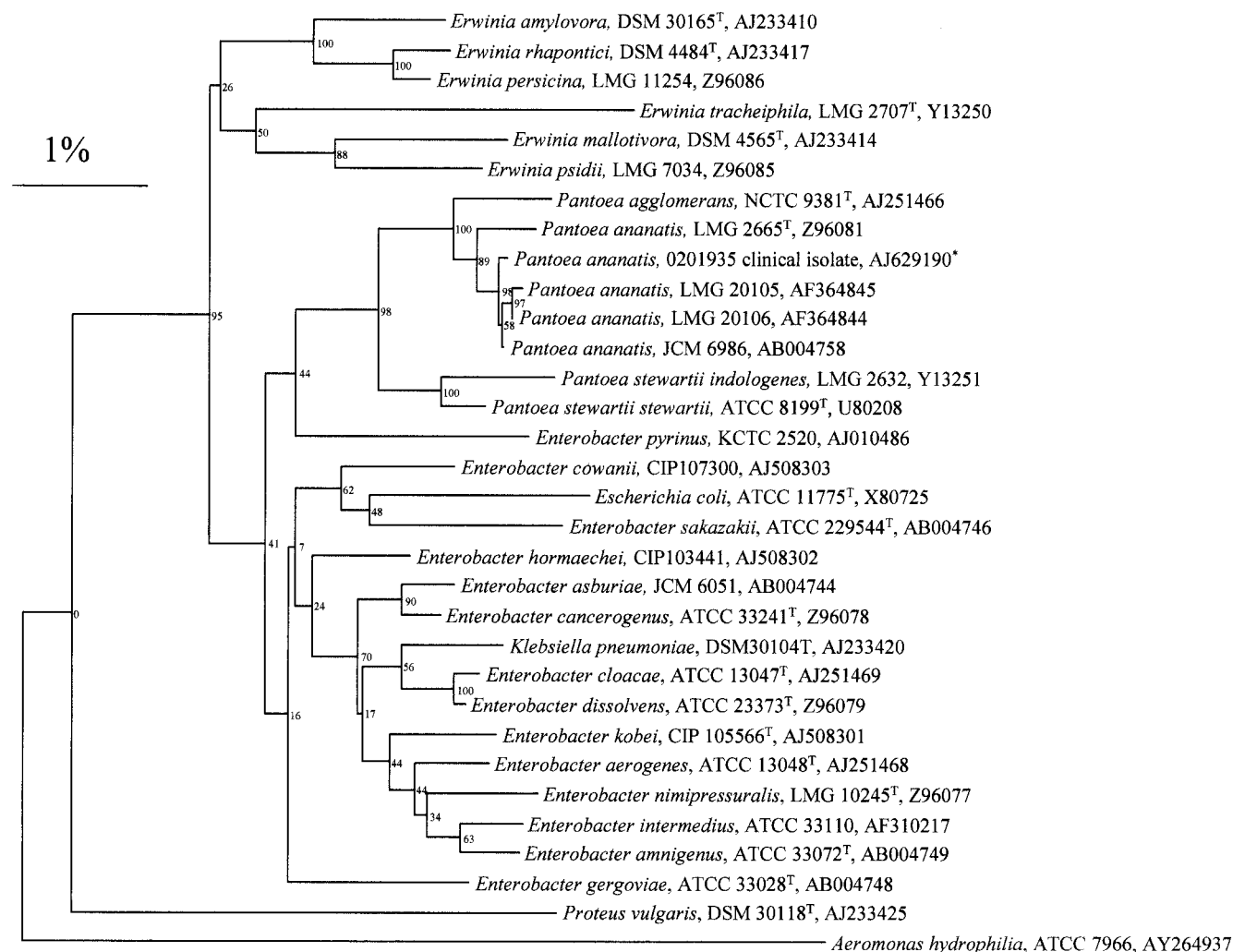


FIG. 1. Dendrogram of the 16S rDNA sequences of the obtained clinical strain and related species. A rooted tree resulting from a neighbor-joining bootstrap analysis (100 replications) of 16S rRNA gene sequences from the clinical isolate and related species published in GenBank, with *Aeromonas hydrophila* as an outgroup, is shown. The bar indicates 1% sequence dissimilarity. *, sequence obtained during this study.

143.5, 166.3, 197.7, 258.3, and 388.8 bp), the only member of the genus with some clinical importance, showing that the two species can be differentiated easily from each other by means of tRNA-PCR. tRNA-PCR of the type strain (LMG 2665^T), carried out later, showed a fingerprint identical to that obtained for the clinical isolate.

Subsequent extensive biochemical identification by conventional methods was consistent with the sequence-based identification as *P. ananatis*. The colonies had a yellow pigment. The cells were motile, oxidase negative, and catalase positive and they fermented glucose, lactose, sorbitol, raffinose, D-arabitol, and alpha-methyl-D-mannoside but not adonitol and dulcitol. The Voges-Proskauer test, indole production, citrate, and gelatin were positive. Urease and hydrogen sulfide production, malonate, and Tween-esterase were negative. *o*-Nitrophenyl-β-D-galactopyranoside, *N*-acetyl-glucosaminidase, beta-xylosidase, and pyrrolidonyl aminopeptidase were positive, but beta-glucuronidase was negative. Using the Kirby-Bauer disk-diffusion method, the strain was found to produce large inhibition zones around disks with ampicillin, cotrimox-

azol, cefuroxime, gentamicin, furadantin, temocillin, and quinolones. Therefore, the results were interpreted so as to suggest susceptibility to these antibiotics.

The species of the genus *Pantoea*, i.e., *P. agglomerans*, *P. ananatis*, *Pantoea citrea*, *Pantoea dispersa*, *Pantoea punctata*, *Pantoea stewartii*, and *Pantoea terrea*, are usually isolated from soil, fruit, and vegetables. Some are phytopathogens, like *P. stewartii* subsp. *stewartii*, which causes Stewart's wilt of sweet corn, and *P. citrea*, which is the causative agent of pink disease of pineapple. Others, like *P. agglomerans*, *P. ananatis*, and *P. dispersa*, can be used as biocontrol agents. For example, *P. dispersa* detoxifies the albicidin produced by *Xanthomonas albilineans* and as such has been proposed for biocontrol against leaf scald disease in highly susceptible sugar cane cultivars.

Only a limited number of clinical cases with bacteria belonging to this genus have been described. A single clinical case with *P. dispersa* was described recently (8). A case of *P. agglom-*

merans spondylodiscitis was also described (7). Among the species of this genus, *P. agglomerans* is most commonly isolated in hospitals.

This is to our knowledge the first report of a clinical case with *P. ananatis*, an organism that was given its species epithet *ananas* because it was considered to be a cause of rot of *Ananas comosus* fruitlets, although this has been questioned (5). Strains of the same species that were formerly classified as a separate species (*Erwinia uredovora*) attack the uredia of the mold and wheat pathogen *Puccinia graminis*. It is also known as an "ice nucleation-active" bacterium, which is used in pest control, lowering the cold resistance when colonizing the gut of some insect larvae (11). This species belonged formerly to the genus *Erwinia* but was reclassified with the former *Enterobacter agglomerans* into the new genus *Pantoea* (3–5).

P. ananatis can be identified genotypically by tRNA-PCR and by 16S rDNA sequencing. *P. ananatis* can be differentiated from *P. agglomerans* by indole production and, in the vast majority of the strains, by acid production from sorbitol, raffinose, and α -methyl-D-mannoside. Differentiation should also be made from other yellow-pigmented *Enterobacteriaceae* encountered in clinical samples. *P. ananatis* can be distinguished from *Leclercia adecarboxylata*, *Escherichia hermannii*, and *Escherichia vulneris* by its positive Voges-Proskauer reaction and from *Enterobacter sakazakii* by the lack of ornithine decarboxylase and arginine dihydrolase and by acid production from D-arabitol.

Nucleotide sequence accession number. The DNA sequence of the clinical isolate obtained in this study was deposited in GenBank under accession number AJ629190.

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