Stenotrophomonas acidaminiphila sp. nov., a strictly aerobic bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor

Essokazi A. Assih,[1] Aboubakar S. Ouattara,[1] Sébastien Thierry,[2,3] Jean-Luc Cayol,[4] Marc Labat[4] and Hervé Macarie[2,3]

[1] Département de Biochimie-Microbiologie, Faculté des Sciences et Techniques, Université de Ouagadougou, 03 BP 7021, Ouagadougou 03, Burkina Faso

[2] Departamento de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa, Avenida Michoacán y la Purisima s/n, Col. Vicentina, 09340 México DF, Mexico

[3] Institut de Recherche pour le Développement (IRD), Cicerón 609, Col. Los Morales, 11530 México DF, Mexico

[4] Laboratoire de Microbiologie IRD, IFR-BAIM, Universités de Provence et de la Méditerranée, ESIL case 925, 163 avenue de Luminy, 13288 Marseille cedex 9, France

Author for correspondence: Aboubakar S. Ouattara. Tel: +226 33 73 73. Fax: +226 33 73 73. e-mail: ouattabs@univ-ouaga.bf

Two of several strictly aerobic, mesophilic bacteria isolated from a lab-scale upflow anaerobic sludge blanket (UASB) reactor treating a petrochemical wastewater, strains AMX 17 and AMX 19^T, were subjected to detailed taxonomic study. Cells were Gram-negative, motile, non-sporulating, straight to curved rods with a polar flagellum. The isolates exhibited phenotypic traits of members of the genus *Stenotrophomonas*, including cellular fatty acid composition and the limited range of substrates that could be used. Sugars and many amino acids were utilized. Antibiotic susceptibility and physiological characteristics were determined. The DNA base composition was 66·9 mol% G+C. Phylogenetic analysis revealed that the nearest relatives were *Stenotrophomonas maltophilia* LMG 11114, *Stenotrophomonas nitritireducens* DSM 12575^T and *Pseudomonas pictorum* ATCC 23328^T (similarity of 98·1–98·8%). *Xanthomonas* species, *S. maltophilia* LMG 958^T and *Stenotrophomonas africana* CIP 104854^T showed high 16S rRNA sequence similarities (96·4–97·3%). The high similarity found in cellular fatty acid profiles and identical partial 16S rRNA sequences (500 bp) for strains AMX 17 and AMX 19^T indicate that they belong to the same species. DNA–DNA hybridizations revealed respectively 26·7, 31, 65·8 and 43·6% homology between isolate AMX 19^T and *S. africana* CIP 104854^T, *S. maltophilia* CIP 60.77^T, *S. nitritireducens* DSM 12575^T and *P. pictorum* ATCC 23328^T. These results allow the proposal of strain AMX 19^T (= DSM 13117^T = ATCC 700916^T = CIP 106456^T) as representative of a novel species of the genus *Stenotrophomonas*, with the name *Stenotrophomonas acidaminiphila* sp. nov.

Keywords: polyphasic taxonomy, cellular fatty acids, amino acids, anaerobic reactor, *Xanthomonas*

INTRODUCTION

Anaerobic digesters are complex, man-made biotopes, used for treatment of wastewater, sludge and organic solids, where both strictly anaerobic and aerobic bacteria can co-exist (Guyot *et al.*, 1994; Noeth *et al.*, 1988; Toerien & Hattingh, 1969). Surveys of bacterial population types have revealed the presence, in these ecosystems, of aerobic, non-sporulating, Gram-negative, flagellated, chemo-organotrophic proteobacteria that possess a strictly respiratory energy-yielding metabolism (Britz *et al.*, 1994; Hakulinen *et al.*, 1985; Ng *et al.*, 1994; Toerien & Hattingh, 1969). A wide diversity of genera, including *Stenotrophomonas*, are members of this heterogeneous group with versatile metabolisms. Initially containing a single species, *Stenotrophomonas maltophilia*, the number of species of the genus *Stenotrophomonas* has increased to three with the recent characterization and description of *Stenotrophomonas africana* (Drancourt *et al.*, 1997) and *Stenotrophomonas nitritireducens* (Finkmann *et*

Abbreviations: CFA, cellular fatty acid; UASB, upflow anaerobic sludge blanket.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AMX 19^T is AF273080.

al., 2000). The original member of the genus, *S. maltophilia*, originally isolated from human pleural fluid and named '*Bacterium bookerii*', was validly described as *Pseudomonas maltophilia* (Hugh & Ryschenkow, 1961) before being renamed *Xanthomonas maltophilia* on the basis of quinone type, cellular fatty acid (CFA) composition, enzyme characterization and DNA–rRNA hybridization results (Swings *et al.*, 1983). However, the affiliation of *P. maltophilia* to the genus *Xanthomonas* was subject to criticism (Van Zyl & Steyn, 1992), and a further reclassification of *X. maltophilia* to *S. maltophilia* was proposed (Palleroni & Bradbury, 1993).

This species has been the object of several taxonomic studies (Hauben *et al.*, 1999; Moore *et al.*, 1997; Palleroni & Bradbury, 1993; Stanier *et al.*, 1966; Van den Mooter & Swings, 1990; Vauterin *et al.*, 1995; Wang *et al.*, 1998). *Stenotrophomonas* species are common inhabitants of a wide variety of natural and artificial environments such as water, sediments, plant rhizospheres, corroded metal surfaces, waste-gas biofilters, aquaculture tanks, oilfields, sewage and anaerobic reactors (Aznar *et al.*, 1992; Boonchan *et al.*, 1998; Borowicz *et al.*, 1995; Britz *et al.*, 1994; Finkmann *et al.*, 2000; Hauben *et al.*, 1999; Juhnke *et al.*, 1987; Lambert *et al.*, 1990; Leifert & Waites, 1992; Wallace *et al.*, 1994; Wilkinson *et al.*, 1994) and can be opportunistic human pathogens (Drancourt *et al.*, 1997; Denton & Kerr, 1998).

In an attempt to determine the role of strictly aerobic bacteria in anaerobic digesters, enumeration and identification of these micro-organisms was performed from a laboratory-scale upflow anaerobic sludge blanket (UASB) reactor fed with the wastewater of a petrochemical company producing purified terephthalic acid (1,4-benzenedicarboxylic acid). Several strains were isolated and subjected to identification by classical biochemical character determination, CFA composition analysis and/or partial 16S rRNA sequence analysis. Most of the isolates could be identified accurately by the set of taxonomic tools but some were not readily identifiable. Among this last group of micro-organisms, several strains, AMX strains 15, 17, 18, 19^T and 312, could be arranged in the same cluster based on their CFA profiles. Strains AMX 17 and AMX 19^T were then subjected to more detailed taxonomic study. We report the characterization of strains AMX 17 and AMX 19^T as a novel species of the genus *Stenotrophomonas*, *Stenotrophomonas acidaminiphila* sp. nov., with strain AMX 19^T (= DSM 13117^T = ATCC 700916^T = CIP 106456^T) as the type strain.

METHODS

Source of organisms. Strains AMX 17 and AMX 19^T were isolated from the anaerobic sludge of a laboratory-scale UASB reactor using R2A medium (Oxoid). The reactor, fed with settled, genuine, terephthalic-acid-plant wastewater supplemented with nitrogen, phosphorous, sulfur and trace metals, was operated at a constant organic loading rate of 2·25 g COD $l^{-1}_{reactor}$ day^{-1} [0·1 g COD (g VSS)$^{-1}$ day^{-1}] (COD, chemical oxygen demand; VSS, volatile suspended solids) and a hydraulic retention time of 2 days. The temperature *in situ* averaged 33 °C and the pH was near 7·4. The main organic compounds present in the wastewater were acetic, benzoic, *p*-toluic (4-methylbenzoic), trimellitic (1,2,4-benzenetricarboxylic) and terephthalic acids, as well as 4-carboxybenzaldehyde (4-formylbenzoic acid) and ethylene glycol (Fajardo *et al.*, 1997). By the time of isolation, the reactor was fed with non-sterilized wastewater and could be considered in stationary phase, since it presented constant biogas production (1·08 l day^{-1} at 1 atm, 0 °C) and COD removal (80 ± 6 %). Purifications were performed by streaking colonies onto Petri dishes containing medium R2A or plate count agar (Difco). The purity of isolates was checked in a complex medium containing 0·5 % yeast extract, 0·5 % peptone, 0·5 % Bio-trypticase, 0·5 % Casamino acids and 0·25 % glucose. The culture was examined microscopically after 1–3 weeks of incubation. *S. maltophilia* CIP 60.77^T and *S. africana* CIP 104854^T were obtained from the Collection de l'Institut Pasteur (CIP), *S. nitritireducens* DSM 12575^T was from the DSMZ and *P. pictorum* ATCC 23328^T was from the ATCC.

Media and culture conditions. With the exception of the isolation and purification procedures, all procedures were performed in liquid medium using standard techniques for cultivation of strict aerobes. For nutritional tests, strains were grown on a basal medium containing (l^{-1}): 0·25 g KH_2PO_4, 0·30 g NH_4Cl, 1 g NaCl, 0·50 g KCl, 0·40 g $MgCl_2.6H_2O$, 0·16 g $CaCl_2.2H_2O$ and 0·10 g Casamino acids. Media were dispensed into tubes or flasks and sterilized at 120 °C for 18 min. Just before inoculation, substrates (sugars, organic acids or amino acids) were supplied from filter-sterilized stock solutions in order to reach a final concentration of 10 mM, except for the aromatic amino acids, which were added at 1 g l^{-1}, and the racemic mixtures, which were prepared at 20 mM.

Determination of optimal growth conditions (pH, temperature) was conducted in a rich culture medium (basal medium supplemented with 5 g Casamino acids and 2 g yeast extract l^{-1}). The pH was fixed at 7 when testing temperature and the temperature at 35 °C when testing pH. Growth was monitored by measuring the optical density at 580 nm. The ranges tested for growth were pH 5·0–9·5 and 4–50 °C.

General phenotypic characteristics. Phenotypic characterization of isolate AMX 19^T was based on Gram staining, motility, respiratory, catalase and oxidase tests (Diagnostics Pasteur). Further biochemical analysis was performed by inoculating API 20 NE and API 50 CH strips (bioMérieux) according to the manufacturer's instructions. The cell morphology was determined from direct observations of fresh cultures using a Nikon phase-contrast microscope or exponentially grown cells negatively stained with 1 % sodium phosphotungstic acid (pH 7·2) with a Hitachi model H600 transmission electron microscope operated at an accelerating voltage of 75 kV. The presence, number and position of flagella were determined both by flagellar staining and by electron microscopy.

Antibiogram. Susceptibility of strain AMX 19^T to 15 antibiotics was tested by Pasteur Cerba (Paris, France) using the Kirby–Bauer disk-diffusion method (Bauer *et al.*, 1966) on Mueller–Hinton solid medium (l^{-1}: 3 g beef infusion, 17·5 g Casamino acids, 1·5 g starch, 17 g agar). Inhibition diameters were recorded after 24 h of incubation at 37 °C

under aerobic conditions. The classification of each strain as sensitive, not sensitive or intermediately sensitive to the antibiotic was done according to the disk manufacturer's instructions (Sanofi Diagnostics Pasteur), based on the directives of the French antibiogram committee (Comité de l'antibiogramme de la Société Française de Microbiologie, 1997). The antibiotics tested correspond to those presently recommended for *Pseudomonas aeruginosa* and other Gram-negative aerobes. They cover the different mechanisms of antibiotic action (inhibition of synthesis of peptidoglycan, proteins, nucleic acids and folate and disruption of membranes).

CFA analysis. The CFA compositions of strains AMX 17 and AMX 19^T and also those of collection strains were determined by Microbial ID, Inc., using the Sherlock Microbial Identification System software (MIDI Inc.). Prior to analysis, reference strains and isolates were cultivated in trypticase soy broth agar at 28 °C for 24 h. CFA were extracted and analysed by following the instructions and standard procedures of Microbial ID, Inc. (Miller, 1982; Sasser, 1990).

Nitrate and nitrite reduction. A preliminary examination of the ability of the strains to reduce nitrate and nitrite was performed by the traditional colorimetric procedure (Smibert & Krieg, 1994). This test consists of the addition, after incubation, to the culture medium supplemented with nitrate or nitrite, of sulfanilic acid and *N*,*N*-dimethyl-1-naphthylamine, which react with nitrite to produce a pink/red coloration. The presence of colour in the medium supplemented with nitrate is indicative of its reduction to nitrite, while an absence of coloration in the medium supplemented with nitrite is indicative of its reduction. In case no colour appeared in the medium supplemented with nitrate, zinc powder, which converts nitrate to nitrite, was also added in order to confirm that nitrate was reduced further than nitrite and did not remain in the medium. The ability of the strain to reduce nitrate was double-checked with the API 20 NE strips inoculated for biochemical analysis. Nitrate and nitrite reduction were later confirmed by inoculating, in each case, three tubes containing 10 ml of the following sterile medium (l^{-1}): 5·1 g Na_2HPO_4, 1·1 g NaH_2PO_4, 5·0 g K_2HPO_4, 0·229 g NH_4Cl; 0·033 g $FeSO_4.7H_2O$ and 3 ml of a trace metal solution (El-Mamouni *et al.*, 1995). $CH_3COONa.3H_2O$ (6·8 g l^{-1}, 50 mM) was used as the carbon source, while nitrate and nitrite were added as $NaNO_3$ and $NaNO_2$ at concentrations of 100 and 150 mM, sufficient to allow the oxidation of all the acetate. At these concentrations, neither nitrate nor nitrite appeared to produce significant inhibition of growth of strain AMX 19^T. The medium was made anoxic by boiling under a flow of carbon dioxide, which also corresponded to the final atmosphere of the tubes. With this mode of preparation, the pH of the medium after inoculation (10% v/v) was around 7. The reduction of nitrate and nitrite was monitored by following their disappearance as well as the formation of N_2O in the gaseous phase and ammonium in the aqueous phase. Molecular nitrogen was not followed, since it was impossible to obtain an N_2-free atmosphere by bubbling CO_2 or other gases (i.e. helium).

DNA base determination. The G+C content of the DNA of strain AMX 19^T was determined by HPLC at the 'DSMZ', with non-methylated λ phage DNA as the reference. Procedures for isolation and determination were from Cashion *et al.* (1977) and Mesbah *et al.* (1989).

16S rRNA sequencing and phylogeny. Extraction of DNA from strains AMX 17 and AMX 19^T, 16S rRNA gene amplification, purification and sequencing were performed by MIDI Labs. The 16S rRNA gene was amplified by PCR from genomic DNA isolated from bacterial colonies. Primers 005F and 1540R (F, forward; R, reverse; Lane, 1991) used for the amplification of the full-length sequence (strain AMX 19^T) correspond to *Escherichia coli* positions 5 and 1540 and primers 005F and 531R, used for the partial, 500 bp sequence (strain AMX 17), correspond to positions 5 and 531. Amplification products were purified using Microcon 100 molecular mass cut-off membranes (Amicon) and checked for quality and quantity by running a portion of the products on an agarose gel. Cycle sequencing of the 16S rRNA amplification products was carried out using AmpliTaq FS DNA polymerase and dRhodamine dye terminators. Excess dye-labelled terminators were removed from the sequencing reactions using Sephadex G-50 spin columns. The products were collected by centrifugation, dried under vacuum and frozen at −20 °C until ready to load. Samples were resuspended in a solution of formamide/blue dextran/EDTA and denatured prior to loading. The samples were electrophoresed on an ABI Prism 377 DNA sequencer. Data were analysed using the PE/Applied Biosystems DNA editing and assembly software. The primers used for sequencing were 005F, 338F, 357R, 515F, 531R, 776F, 810R, 1087F, 1104R, 1174F, 1193R and 1540R. The 16S rRNA gene sequence obtained was aligned manually with reference sequences extracted from the RDP (Maidak *et al.*, 1996) and GenBank databases. Positions of sequence and alignment uncertainty were omitted from the analysis. Pairwise evolutionary distances based on 1432 unambiguous nucleotides were computed by using the method of Jukes & Cantor (1969) and dendrograms were constructed from these distances by the neighbour-joining method. The reliability of the trees was evaluated by the bootstrap procedure. All programs used form part of the PHYLIP package (Felsenstein, 1993).

DNA–DNA hybridization. Spectroscopic DNA–DNA hybridization was performed at the DSMZ. DNA was isolated according to Cashion *et al.* (1977). DNA–DNA hybridizations were carried out as described by De Ley *et al.* (1970), with the modifications made by Escara & Hutton (1980) and Huß *et al.* (1983), using a Gilford System model 2600 spectrometer equipped with a Gilford model 2527-R thermoprogrammer and plotter. Renaturation rates were computed using the program TRANSFER.BAS (Jahnke & Bahnweg, 1986; Jahnke, 1992). The hybridization percentages obtained with this technique presented a standard deviation of less than 2·5%. Values close to 70% were re-checked in order to confirm the results.

Analytical techniques. Substrate concentrations were determined by HPLC (Spectra Series 100 model; Thermo Separation Products) connected to a differential refractometer (RID-6A Shimadzu). The mobile phase was 2·5 mM H_2SO_4 at a flow rate of 0·6 ml min^{-1} and the column temperature was 35 °C. The volume of the injection loop was 20 µl. Peak analysis was performed using a CR-6A Shimadzu integrator. Amino acids were assayed using an Aminex HPX-87X column [300 × 7·8 (i.d.) mm] (Bio-Rad) and carbohydrates, organic acids, alcohols and volatile fatty acids with an ORH-801 column [300 × 6·5 (i.d.) mm] (Interaction Chemicals, Inc.). The concentrations of nitrate and nitrite were measured with a capillary ion analyser (Waters 4000; Millipore) according to Gomez *et al.* (1996). N_2O was detected with a Varian 3350 GC equipped with a thermal conductivity detector and a stainless steel column [2000 × 3·2 (i.d.) mm] packed with Porapak Q. Separation was obtained

with the following conditions: injector temperature, 100 °C; column temperature, 35 °C; detector temperature, 110 °C; filament temperature, 135 °C; carrier gas, helium at 16 ml min^{-1}. Gas sampling was performed with a pressure-lock syringe and a volume of 0·1 ml was injected. The concentration of ammonium was determined by the colorimetric method of Nessler as described by Daniels *et al.* (1994).

RESULTS

Morphology, growth, metabolic properties and antibiotic susceptibility

After 2–10 days at 30 °C, strains AMX 17 and AMX 19^T formed yellow, circular colonies on trypticase soy agar. Growth was not accompanied by odour. Cells of isolate AMX 19^T were straight to curved rods, highly motile, that stained Gram-negative and possessed monotrichous polar flagellation. Spore formation was not observed. The cells occurred singly or in pairs and were 0·5 × 1·5–2·5 µm in size. Tests for catalase, oxidase, Tween 80 esterase, nitrite and nitrate reductases and aesculin hydrolysis were positive while tests for indole, DNase, lysine and ornithine decarboxylases, *o*-nitrophenyl *β*-D-galactopyranoside and *p*-nitrophenyl *β*-D-galactopyranoside, arginine dihydrolase as well as urease, Simmons' citrate, amylase and proteolysis were negative. Strain AMX 19^T was strictly aerobic, as shown by the absence of growth after 1 month of incubation at 35 °C in an anaerobic jar on R2A medium. However, growth was possible under anoxic conditions, coupled to nitrate and nitrite consumption, with acetate as the sole carbon source. Since ammonium accumulation was not observed in the medium and only traces of N_2O were detected (data not shown) in the gas phase of old cultures (40 days incubation), we assumed that strain AMX 19^T was able to perform the complete reduction of both compounds to N_2. Growth was obtained when organic acids, sugars, amino acids, Casamino acids and peptone from casein were used as carbon sources (Table 1). Fastest growth and greatest biomass recovery were observed with Casamino acids and peptone from casein. Degradation of tyrosine and phenylalanine was accompanied by a pink coloration of the culture medium. Benzoate was initially used as a carbon source. This ability was, however, lost quickly upon subculturing on R2A agar or any rich medium. Susceptibility of the growth of isolate AMX 19^T to antibiotics was observed with all the aminoglycosides, fluoroquinolones, polypeptides and sulfamides tested, which are characterized by modes of action not based on the inhibition of peptidoglycan synthesis. In this latter group of antibiotics, susceptibility to the cephems and penicillins studied was variable, while resistance was observed with the only carbapenem examined (Table 2).

Growth was observed above 20 °C (the lower limit was between 4 °C, at which no growth occurred, and 20 °C, but was not determined with precision) and below 42 °C, with an optimum at 30–35 °C. The pH range of growth was 5·0–9·0, with optimal growth at pH 6·0–7·0.

CFA analysis

The three CFA (11:0 iso; 11:0 iso 3OH and 13:0 iso 3OH) identified by Yang *et al.* (1993) as characteristic of the genera *Stenotrophomonas* and *Xanthomonas* were detected in the CFA patterns of AMX 17 and AMX 19^T (Table 3). Comparison of the CFA profiles of these strains to those of all *Stenotrophomonas* species described so far and 11 *Xanthomonas* species present in the Microbial ID database by unweighted arithmetic average clustering showed that these micro-organisms clustered with *S. nitritireducens* DSM 12575^T but separately from the other *Stenotrophomonas* species and from the *Xanthomonas* species (Fig. 1). In the same analysis, strains AMX 17 and AMX 19^T linked together at a Euclidian distance of 3, which indicates that the two organisms have very similar CFA patterns. Their CFA profiles showed several differences from those of *S. maltophilia* CIP 60.77^T, *S. africana* CIP 104854^T and *S. nitritireducens* DSM 12575^T (Table 3). The main differences from these species are: (i) the presence (even if only as traces) of 10:0 iso, 12:0 iso, 13:0 anteiso, 14:1*ω*5*c*, 15:1*ω*6*c* and 16:1 iso H fatty acids; (ii) larger amounts of 14:0 iso and particularly 16:0 iso; and (iii) smaller amounts of 15:0 anteiso. The 17:0 cyclo fatty acid reported as distinctive for *S. nitritireducens* by Finkmann *et al.* (2000) was also absent from both strains, while they possessed 10:0, 18:1*ω*9*c* and 18:1*ω*7*c*, not detected in the latter species. The differences observed between our results and CFA patterns reported previously for *S. maltophilia* (Yang *et al.*, 1993) and *S. nitritireducens* (Finkmann *et al.*, 2000) are probably the result of different times (48 or 72 h instead of 24 h) and/or temperatures (25 against 28 °C) of incubation.

Genotypic analysis

The G+C content of isolate AMX 19^T was 66·9 ± 0·5 mol% (three determinations). A total of 1542 positions of its 16S rRNA gene were sequenced. Phylogenetic analysis revealed that the closest relatives were *S. maltophilia* LMG 11114, *S. nitritireducens* DSM 12575^T and *P. pictorum* ATCC 23328^T, with similarity levels of 98·8, 98·6 and 98·0 %, respectively. *S. africana* CIP 104854^T, *Xanthomonas arboricola* LMG 747^T, *Xanthomonas axonopodis* LMG 538^T and *Xanthomonas campestris* LMG 726 were the next nearest relatives, with similarity values of 96·4–97·3 % (Fig. 2). The type strain of *S. maltophilia*, LMG 958^T, presented only 96·7 % similarity. No differences were observed between the 500 bp sequenced from strain AMX 17 and the corresponding sequence from strain AMX 19^T. DNA–DNA hybridization revealed 26·7, 31·0, 43·6 and 65·8 % similarity, respectively, between isolate AMX 19^T and *S. africana* CIP 104854^T, *S. maltophilia* CIP 60.77^T, *P. pictorum* ATCC 23328^T and *S. nitritireducens* DSM 12575^T. The level of DNA hybridization between the type strains of *S. africana*

Table 1. Substrate utilization by strain AMX 19T

Utilization is scored as: +, utilized; +*, utilized with slight increase in OD; (+), slow and poor use without increase in OD; (v), variable; −, not utilized.

Compound	Utilization
Amino acids and related compounds	
DL-Alanine	+
L-Arginine	+
L-Asparagine	+
DL-Aspartate	+
L-Cysteine	+
L-Glutamate	+
L-Glutamine	+
DL-Histidine	+
L-Isoleucine	+
DL-Leucine	+
L-Phenylalanine	+
L-Proline	+
L-Serine	+
DL-Threonine	+
DL-Tyrosine	+
DL-Valine	+
Glycine	−
L-Lysine	−
L-Methionine	−
D-Ornithine	−
DL-Tryptophan	−
Casamino acids	+
Peptone from casein	+
Sugars and related compounds	
N-Acetylglucosamine	+
Maltose	+
D-Mannose	+
D-Cellobiose	−
Amygdalin	−
D-Arabinose	−
L-Arabinose	−
Arbutin	−
D-Fructose	+
D-Fucose	−
L-Fucose	−
D-Galactose	−
β-Gentiobiose	−
D-Glucose	+
Methyl *β*-D-glucoside	−
Glycogen	−
Inulin	−
Lactose	−
D-Lyxose	−
Methyl α-D-mannoside	−
Melezitose	−
Melibiose	−
D-Raffinose	−
Rhamnose	−
Ribose	−
Salicin	−
L-Sorbose	−
Starch	−
Sucrose	−
D-Tagatose	−
Trehalose	−
D-Turanose	−
D-Xylose	−
L-Xylose	−
Methyl *β*-xyloside	−
Organic acids	
Acetate	+
Crotonate	+*
Fumarate	+
DL-Lactate	+
Pyruvate	+
Succinate	+ (v)
Adipate	−
Butyrate	−
Caprate	−
Citrate	−
Gluconate	−
2-Ketogluconate	−
5-Ketogluconate	−
Malate	−
Propionate	−
Alcohols	
Adonitol	−
D-Arabitol	−
L-Arabitol	−
Dulcitol	−
Erythritol	−
Ethanol	−
Ethylene glycol	−
Inositol	−
Glycerol	−
D-Mannitol	−
Propan-1-ol	−
Sorbitol	−
Xylitol	−
Aromatic compounds	
Benzoate	− (v)
4-Carboxybenzaldehyde	−
o-Phthalate	−
Phenylacetate	−
p-Methylbenzoate	−
Terephthalate	−
Trimellitate	−

(CIP 104854T) and *S. maltophilia* (60.77T) found during this study was 65·9%, significantly different from the value of 35% reported by Drancourt *et al.* (1997), which led them to propose *S. africana* as a novel species. This difference is probably due to the fact that the S1 nuclease (TCA) hybridization technique employed by these authors is known to produce

Table 2. Action of antibiotics towards strain AMX 19^T

Sensitivity: +, sensitive; −, not sensitive; ±, intermediate sensitivity. For the amoxicillin + clavulanic acid test, the disk charge with the two compounds was 20 and 10 μg. For the piperacillin + tazobactam test, it was 75 and 10 μg. MIC_{eq}, Equivalent minimum inhibitory concentration according to the Comité de l'antibiogramme de la Société Française de Microbiologie (1997). The reported MIC_{eq} for piperacillin and piperacillin + tazobactam correspond to those recommended for *Pseudomonas aeruginosa*.

Antibiotic	Sensitivity	MIC_{eq} (mg l^{-1})
Aminoglycosides (inhibition of protein synthesis)		
Amikacin (30 μg)	+	⩽ 8
Gentamicin (10 UI)	+	⩽ 4
Netilmicin (30 μg)	+	⩽ 4
Tobramycin (10 μg)	+	⩽ 4
Fluoroquinolones (inhibition of nucleic acid synthesis)		
Ciprofloxacin (5 μg)	+	⩽ 1
Ofloxacin (5 μg)	+	⩽ 1
Polypeptides (disruption of cell membranes)		
Colistin (300 UI)	+	⩽ 2
Sulfonamides + diaminopyrimidines (inhibition of folate synthesis)		
Trimethoprim + sulfamethoxazole (1·25 + 23·75 μg)	+	⩽ 2/38
Carbapenems (inhibition of peptidoglycan synthesis)		
Imipenem (10 μg)	−	> 8
Cephems (inhibition of peptidoglycan synthesis)		
Cefalotin (30 μg)	−	> 32
Cefotaxime (30 μg)	−	> 32
Ceftazidime (30 μg)	+	⩽ 4
Penicillins (inhibition of peptidoglycan synthesis)		
Amoxicillin (25 μg)	−	> 16
Amoxicillin + clavulanic acid	−	> 16
Piperacillin (75 μg)	+	⩽ 16
Piperacillin + tazobactam	+	⩽ 16
Ticarcillin (75 μg)	±	⩾ 16–64

rather low binding values compared with the spectrophotometric method (Hauben *et al.*, 1999).

DISCUSSION

Identification

Strains AMX 17 and AMX 19^T exhibited very high similarity in their CFA profiles and identical partial 16S rRNA sequences (500 bp); we therefore assumed that they belong to the same species, and strain AMX 19^T was chosen arbitrarily as the type strain. The phylogenetic analysis disclosed without doubt that strain AMX 19^T is affiliated to the family *Xanthomonadaceae* of the γ-subclass of the *Proteobacteria* and, within this family, to the genera *Stenotrophomonas* and *Xanthomonas*. This is in agreement with its CFA profile and G + C content and the fact that it is a strictly aerobic, Gram-negative, non-sporulating, motile, straight to curved, rod-shaped bacteria (Palleroni & Bradbury, 1993; Vauterin *et al.*, 1995). Other phenotypic traits, such as testing positive for oxidase, the presence of a nitrate reductase and an optimum growth temperature over 30 °C indicate that strain AMX 19^T cannot be a member of *Xanthomonas* and that it is more likely to be a member of *Stenotrophomonas*, despite its monotrichous flagellation. In accordance with these phenotypic characters, analysis of the 16S rRNA sequence of strain AMX 19^T revealed that the known *Stenotrophomonas* species were its closest relatives together with *P. pictorum* ATCC 23328^T (Fig. 2). As strain AMX 19^T shares only 96·7 and 96·4 % sequence similarity with *S. maltophilia* LMG 958^T and *S. africana* CIP 104854^T, it cannot be a member of either of these species (Stackebrandt & Goebel, 1994). The DNA–DNA hybridization values of strain AMX 19^T with *S. maltophilia* CIP 60.77^T (31 %) and *S. africana* CIP 104854^T (26·7 %), which were significantly below the 70 % proposed by Wayne *et al.* (1987), corroborate this result. It is interesting to note that, in contrast to *S. africana* CIP 104854^T and *S. maltophilia* LMG 958^T or LMG 11114, strain AMX 19^T was never identified as *S. maltophilia* by the MIDI fatty acid identification system or upon use of classical biochemical identification kits such as API 20 NE strips (Drancourt *et al.*, 1997; Hauben *et al.*, 1999; this study). This attests to the fact that AMX 19^T is phenotypically distinct from these species and that it can be distinguished from them easily by simple, commonly available identification tools. The 16S rRNA phylogenetic analysis did not, however, allow us to discriminate strain AMX 19^T at the species level from *P. pictorum* ATCC 23328^T (98·01 % similarity) or *S. nitritireducens* DSM 12575^T (98·6 % similarity). The DNA–DNA reassociation experiments performed to resolve the phylogenetic relationship of these bacteria demonstrated without ambiguity that AMX 19^T cannot be affiliated to *P. pictorum* (hybridization of only 43·6 %). This is consistent with the fact that, in the dendrogram constructed from the CFA patterns, *P. pictorum* linked with strain AMX 19^T at a Euclidian distance of more than 15, which is much higher than the usual cut-off limit between species (Fig. 1). The genomic information obtained during this study, combined with comparative phenotypic analysis done by others (Van den Mooter & Swings, 1990; Oyaizu & Komagata, 1983), suggests that *P. pictorum* should be affiliated to the genus *Stenotrophomonas*. A DNA–DNA hybridization value below 70 % was also observed between *S. nitritireducens* DSM 12575^T and AMX 19^T. Since this value (65·8 %) is close to 70 %, one could argue that this is not sufficient to separate the two strains in different species, particularly when one considers that the type species of *Stenotrophomonas* (*S. maltophilia*), while phenotypically very homogeneous, appears to be characterized by significant genomic diversity (16S rRNA similarity from 91·6 to 99·7 %, DNA–DNA hybridization from 5 to 95 %; Hauben *et al.*, 1999). Nevertheless, in the case of AMX

Table 3. Fatty acid profiles of strains AMX 19T and AMX 17 compared with those of *Stenotrophomonas* species

Values are percentages of total fatty acids.

Fatty acid	AMX 19T	AMX 17	*S. nitritireducens* DSM 12575T	*S. maltophilia* CIP 60.77T	*S. africana* CIP 104854T
9:0	0·03				
10:0 iso	0·97	1·38			
10:0	0·24	0·26		0·70	0·60
11:0 iso	4·06	4·29	5·05	3·43	3·11
11:0 anteiso	0·30	0·34	0·39		
10:0 3OH	0·04			0·22	0·21
12:0 iso	0·14	0·24			
11:0 iso 3OH	1·84	2·03	2·43	1·74	1·58
11:0 3OH	0·10	0·12	0·33		
13:0 iso	0·60	0·71	0·86	0·73	0·66
13:0 anteiso	0·06	0·11			
12:0 iso 3OH	1·85	2·22	1·45		
12:0 3OH	0·60	0·74	1·24	3·01	3·01
14:0 iso	6·59	8·68	3·52	0·54	0·86
14:1ω5*c*	0·16	0·2			
14:0	0·92	0·97	1·37	4·44	4·32
13:0 iso 3OH	1·26	1·62	2·18	3·54	3·22
13:0 2OH	0·23	0·29	0·57	0·25	0·29
15:1 iso F	2·75	2·89	2·67	1·30	1·16
15:0 iso	35·95	33·64	35	36·65	36·68
15:0 anteiso	5·19	5·02	8·23	6·96	8·78
15:1ω8*c*	0·10				
15:1ω6*c*	0·39	0·29			
15:0	0·63	0·47	1·83	0·33	0·55
16:1 iso H	0·13	0·19			
16:0 iso	10·20	10·50	5·38	0·62	1·15
16:1ω9*c*	0·87	0·79	0·97	3·72	3·33
16:0	2·28	2·14	4·80	6·51	6·48
15:0 iso 3OH	0·10				
Iso 17:1ω9*c*	12·72	12·18	12·87	4·51	4·38
17:0 iso	2·02	1·55	3·33	3·10	2·96
17:0 anteiso				0·17	0·24
17:1ω8*c*	0·47	0·38	1·02		0·18
17:0 cyclo			1·23		
17:0	0·11				
18:1ω9*c*	0·11	0·17		1·43	1·31
18:1ω7*c*	0·10	0·17		0·77	0·78
18:0		0·21	0·23		
19:0 iso				0·40	0·32

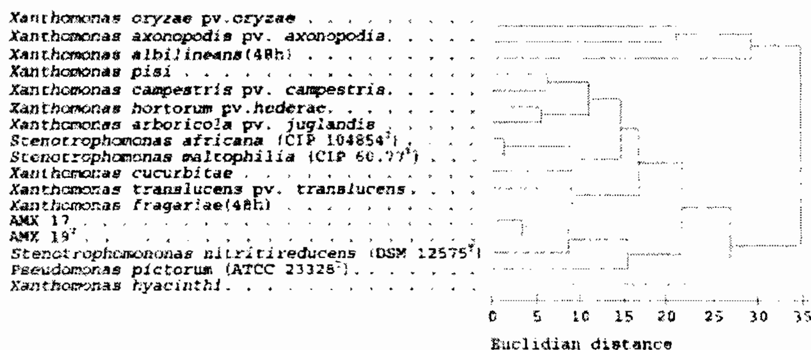

Fig. 1. Dendrogram generated from CFA compositions showing the relationship of strains AMX 17 and AMX 19T with 11 *Xanthomonas* species and all *Stenotrophomonas* species.

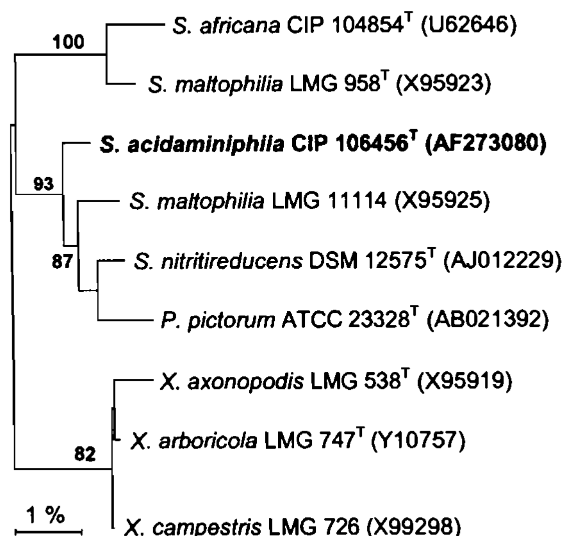

Fig. 2. Phylogenetic dendrogram showing the position of strain AMX 19^T (= CIP 106456^T) among representative members of the genus *Stenotrophomonas* and other closely related *Proteobacteria*. Bar, 1 nucleotide substitution per 100 nucleotides. Percentages at nodes correspond to bootstrap values based on 1000 resamplings. Only values greater than 80% were considered significant and therefore are reported.

19^T, the genomic difference from *S. nitritireducens* DSM 12575^T is clearly correlated by at least 17 phenotypic differences, including different spectra of substrates used (Table 4), a different CFA pattern (Table 3), the presence of a nitrate reductase, the absence of N_2O formation upon NO_3 reduction and the ability to hydrolyse aesculin.

On the basis of the above-mentioned results, we propose that strains AMX 19^T (= DSM 13117^T = ATCC 700916^T = CIP 106456^T) and AMX 17 represent a novel species of genus *Stenotrophomonas*, *Stenotrophomonas acidaminiphila* sp. nov.

Ecological aspects

The isolation of strain AMX 19^T, a strict aerobe, from the sludge of an anaerobic reactor raises the question of whether such a system corresponds to its usual biotope or whether it was found there by accident. At the time of isolation, the anaerobic reactor was fed with non-sterilized wastewater and, as a consequence, strain AMX 19^T could have been introduced to the system in this way. Nevertheless, 3·5 months after switching feeding to sterilized wastewater, strains with CFA profiles identical to AMX 19^T were still found in the reactor at levels as high as 3×10^5 c.f.u. ml^{-1}. Since strain AMX 19^T is unable to sporulate or to produce any form of resistance, its presence in such a biotope for such a long period of time suggests that it was metabolically active even as ultramicrobacteria (Iizuka *et al.*, 1998) and that it is a permanent member of its microflora. Since the wastewater contained a low concentration of nitrate (3·1 mg l^{-1}, 50 μM), survival

Table 4. Characteristics, other than fatty acids, that differentiate strain AMX 19^T from its closest relative, *S. nitritireducens*

Data for *S. nitritireducens* were taken from Finkmann *et al.* (2000), Lipsky & Altendorf (1997) and this study (sensitivity to antibiotics). Characteristics are scored as: + or −, test is positive (negative), a substrate is used (not used) or the strain is sensitive (not sensitive) to the corresponding antibiotic; (+), variable; ±, sensitivity of the strain to the antibiotic is intermediate.

Characteristic	AMX 19^T	*S. nitritireducens* DSM 12575^T
Oxidase	+	−
Aesculin hydrolysis	+	−
Nitrate reductase	+	−
Production of N_2O from nitrite reduction	−	+
Substrate utilization:		
L-Proline	+	−
Maltose	+	−
D-Mannose	+	−
Fumarate	+	−
Propionate	−	+
Pyruvate	+	−
Succinate	(+)	−
Sensitivity to antibiotics:		
Cefotaxime (30 μg)	−	+
Imipenem (10 μg)	−	+
Ticarcillin (75 μg)	±	+
Trimethoprim + sulfamethoxazole (1·25 + 23·75 μg)	+	−

by denitrification was a possibility. This notwithstanding, cultures of strains AMX 19^T with diluted (1:3) wastewater (plus N, P, S, trace elements, 15 g agar l^{-1}), supplemented with $NaNO_3$ to maintain a concentration of 3·1 mg nitrate l^{-1}, always failed to produce colonies in anaerobic jars even after 1 month of incubation at 35 °C. As a consequence, the only logical explanation for the permanence of this strain seems related to the fact that the wastewater was not de-aerated before feeding. Under these conditions, the reactor, even if macroscopically anaerobic ($E_h = -101 \pm 20{\cdot}4$ mV; CH_4 production; no oxygen detected at the reactor outlet), was in reality slightly aerated. Based on the concentration of O_2 in the wastewater (1·6 mg l^{-1}), it can be estimated that the system received 0·8 mg O_2 $l_{reactor}^{-1}$ day^{-1}, which represents less than 0·035% of the organic loading rate, confirming that the greater part of the organic matter was necessarily degraded anaerobically. Strain AMX 19^T could have survived in the system at the expense of acetate or benzoate, which are the two wastewater organic pollutants that it uses as carbon sources, and oxygen, for which it has a high affinity (i.e. K_m for O_2 as low as 8 μg O_2 l^{-1} or 0·25 μM with acetate as carbon source). The significance of the

loss by freshly isolated strains of AMX 19^T of the ability to use benzoate upon subculturing on solid media is not known, but similar results were observed previously for benzoate and hydrocarbons with *S. maltophilia* strains isolated from the soils of rice fields and oil fields (Garcia *et al.*, 1981; Palleroni & Bradbury, 1993). The carbonaceous substrates available to strain AMX 19^T were, however, probably not restricted to those present in the wastewater. For instance, various organic compounds may be released during cell lysis and others, such as exopolymers, excreted by several organisms. These compounds include amino acids and peptides, which have been found as preferred substrates of strain AMX 19^T. Moreover, in contrast to what happens with acetate, AMX 19^T would not have to compete for them with methanogenic bacteria. Since strain AMX 19^T was isolated from the environment, its impact on human health is not known, and it would be interesting to evaluate it, considering that some of its close relatives (*S. maltophilia* and *S. africana*) are recognized opportunistic pathogens. The preliminary information given by the antibiogram suggests, nevertheless, that it is sensitive to a large panel of antimicrobial agents presently available for therapeutics.

Description of *Stenotrophomonas acidaminiphila* sp. nov.

Stenotrophomonas acidaminiphila (a.ci.da.mi.ni.phi'la. N.L. *acidum* acid; N.L. n. *aminum* amine; N.L. fem. adj. *phila* from Gr. adj. *philos* loving; N.L. adj. *acidaminiphila* loving amino acids).

Cells are straight to curved rods, 0·5 µm wide and 1·5–2·5 µm long. Gram-negative, non-sporulating, strictly aerobic bacterium. Motile by one polar flagellum. Pale yellow colonies on common nutritive medium. Tests for catalase, oxidase, aesculin hydrolysis, Tween 80 esterase and nitrite and nitrate reductases are positive. Substrates utilized are listed in Table 1. Benzoate utilization lost upon subculturing. Casamino acids ($0{\cdot}1\ g\ l^{-1}$) are required for growth. Antibiotic susceptibility: amikacin, ceftazidime, ciprofloxacin, colistin, gentamicin, netilmicin, ofloxacin, piperacillin, ticarcillin, trimethropim sulfamethoxazole and tobramycin. Predominant fatty acids are, in order of abundance: 15:0 iso, 17:1 iso$\omega 9c$, 16:0 iso, 14:0 iso, 15:0 anteiso and 11:0 iso. Trace fatty acids that distinguish the species from other *Stenotrophomonas* species are 10:0 iso; 12:0 iso; 13:0 anteiso, 14:1$\omega 5c$, 15:1$\omega 6c$ and 16:1 iso H. The pH range for growth is 5·0–9·0, with optimum growth at pH 6·0–7·0. Temperature range for growth, $<$ 20–42 °C, optimum at 30–35 °C. The DNA G+C content is $66{\cdot}9 \pm 0{\cdot}5$ mol%.

Habitat: isolated from anaerobic sludge of a lab-scale UASB reactor, treating the petrochemical wastewater of a purified terephthalic acid plant in Mexico. The type strain is AMX 19^T (= DSM 13117^T = ATCC 700916^T = CIP 106456^T).

ACKNOWLEDGEMENTS

For the realization of this work, A.S.O. received a postdoctoral fellowship from IRD and E.A. received a scholarship from the Government of Togo. S.T. was supported financially successively by IRD and the Secretaria de Relaciones Exteriores (Foreign Affairs) of Mexico. Thanks are due to Martine Keridjian of the Pasteur Institute for the respective determination of some of the biochemical characteristics and to Nicolas Fortineau of Pasteur Cerba for determining the antibiotic susceptibility of strain AMX 19^T. Thanks are due also to Pierre Thomas for electron microscope facilities, Frédéric Verhe and Jaime Perez Trevilla for technical assistance and Sylvie Escoffier, Marie Laure Fardeau, Jean-Louis Garcia and Pierre Roger for fruitful discussions and support. Special thanks go to Karen Dohrman of Microbial ID Inc., John Bartell of MIDI Labs and Peter Schumann of DSMZ who, besides the respective determination of CFA, 16S rRNA sequences and DNA hybridization, were always ready to give us professional and fine suggestions on the corresponding techniques. Finally, we cannot forget Fernando Varela and Roberto Marcelo, from Tereftalatos Mexicanos SA, who gave us the wastewater and inoculum for the operation of the lab-scale reactor from which strains AMX 17 and 19^T were isolated.

REFERENCES

Aznar, R., Alcaide, E. & Garay, E. (1992). Numerical taxonomy of pseudomonads isolated from water, sediments and eels. *Syst Appl Microbiol* **14**, 235–246.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. & Turk, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* **45**, 493–496.

Boonchan, S., Britz, M. L. & Stanley, G. A. (1998). Surfactant-enhanced biodegradation of high molecular weight polycyclic aromatic hydrocarbons by *Stenotrophomonas maltophilia*. *Biotechnol Bioeng* **59**, 482–494.

Borowicz, J. J., Brishammer, S. & Gerhardson, B. (1995). A *Xanthomonas maltophilia* isolate tolerating up to 1 percentage sodium azide in Tris/HCl buffer. *World J Microbiol Biotechnol* **11**, 236–237.

Britz, T. J., Spangenberg, G. & Venter, C. A. (1994). Acidogenic microbial species diversity in anaerobic digesters treating different substrates. *Water Sci Technol* **30** (12), 55–61.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

Comité de l'antibiogramme de la Société Française de Microbiologie (1997). Communiqué 1997. *Pathol Biol* **45**, 1–12.

Daniels, L., Hanson, R. S. & Phillips, J. A. (1994). Chemical analysis. In *Methods for General and Molecular Bacteriology*, chapter 22, pp. 512–554. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Denton, M. & Kerr, K. G. (1998). Microbiological and clinical aspects of infections associated with *Stenotrophomonas maltophilia*. *Clin Microbiol Rev* **11**, 57–80.

Drancourt, M., Bollet, C. & Raoult, D. (1997). *Stenotrophomonas africana* sp. nov., an opportunistic human pathogen in Africa. *Int J Syst Bacteriol* **47**, 160–163.

El-Mamouni, R., Guiot, S. R., Leduc, R. & Costerton, J. W. (1995). Characterization of different microbial nuclei as potential precursors of anaerobic granulation. *J Biotechnol* **39**, 239–249.

Escara, J. F. & Hutton, J. R. (1980). Thermal stability and renaturation of DNA in dimethyl sulfoxide solutions: acceleration of the renaturation rate. *Biopolymers* **19**, 1315–1327.

Fajardo, C., Guyot, J. P., Macarie, H. & Monroy, O. (1997). Inhibition of anaerobic digestion by terephthalic acid and its anaerobic byproducts. *Water Sci Technol* **36** (6–7), 83–90.

Felsenstein, J. (1993). PHYLIP (Phylogenetic Inference Package) version 3.51c. Distributed by the author. Department of Genetics, University of Washington, Seattle, WA, USA.

Finkmann, W., Altendorf, K., Stackebrandt, E. & Lipski, A. (2000). Characterization of N_2O-producing *Xanthomonas*-like isolates from biofilters as *Stenotrophomonas nitritireducens* sp. nov., *Luteimonas mephitis* gen. nov., sp. nov. and *Pseudoxanthomonas broegbernensis* gen. nov., sp. nov. *Int J Syst Evol Microbiol* **50**, 273–282.

Garcia, J.-L., Roussos, S. & Bensoussan, M. (1981). Taxonomical study of denitrifying bacteria isolated on benzoate in Senegalese rice fields. *Cah ORSTOM Ser Biol* **43**, 13–25 (in French).

Gomez, J., Mendez, R. & Lema, J. M. (1996). The effect of antibiotics on nitrification processes. Batch assays. *Appl Biochem Biotechnol* **57/58**, 869–876.

Guyot, J.-P., Ramirez, F. & Ollivier, B. (1994). Synergistic degradation of acetamide by methanogens and an aerobic Gram-positive rod. *Appl Microbiol Biotechnol* **42**, 452–456.

Hakulinen, R., Woods, S., Ferguson, J. & Benjamin, M. (1985). The role of facultative anaerobic micro-organisms in anaerobic biodegradation of chlorophenols. *Water Sci Technol* **17**, 289–301.

Hauben, L., Vauterin, L., Moore, E. R. B., Hoste, B. & Swings, J. (1999). Genomic diversity of the genus *Stenotrophomonas*. *Int J Syst Bacteriol* **49**, 1749–1760.

Hugh, R. & Ryschenkow, E. (1961). *Pseudomonas maltophilia*, an Alcaligenes-like species. *J Gen Microbiol* **26**, 123–132.

Huß, V. A. R., Festel, H. & Schleifer, K. H. (1983). Studies on the spectrometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Iizuka, T., Yamanaka, S., Nishiyama, T. & Hiraishi, A. (1998). Isolation and phylogenetic analysis of aerobic copiotrophic ultramicrobacteria from urban soil. *J Gen Appl Microbiol* **44**, 75–84.

Jahnke, K.-D. (1992). Basic computer program for evaluation of spectroscopic DNA renaturation data from GILFORD system 2600 spectrometer on a PC/XT/AT type personal computer. *J Microbiol Methods* **15**, 61–73.

Jahnke, K.-D. & Bahnweg, G. (1986). Assessing natural relationships in the basidiomycetes by DNA analysis. *Trans Br Mycol Soc* **87**, 175–191.

Juhnke, M. E., Mathre, D. E. & Sands, D. C. (1987). Identification and characterization of rhizospere-competent bacteria of wheat. *Appl Environ Microbiol* **53**, 2793–2799.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Lambert, B., Meire, P., Joos, H., Lens, P. & Swings, J. (1990). Fast-growing, aerobic, heterotrophic bacteria from rhizosphere of young sugar beet plants. *Appl Environ Microbiol* **56**, 3375–3381.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Leifert, C. & Waites, W. M. (1992). Bacterial growth in plant tissue culture media. *J Appl Bacteriol* **72**, 460–466.

Lipsky, A. & Altendorf, K. (1997). Identification of heterotrophic bacteria isolated from ammonia-supplied experimental biofilters. *Syst Appl Microbiol* **20**, 448–457.

Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. & Woese, C. R. (1996). The Ribosomal Database Project (RDP). *Nucleic Acids Res* **24**, 82–85.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.

Moore, E. R. B., Krüger, A. S., Hauben, L., Seal, S. E., De Baere, R., De Wachter, R., Timmis, K. N. & Swings, J. (1997). 16S rRNA gene sequence analysis and inter- and intrageneric relationships of *Xanthomonas* species and *Stenotrophomonas maltophilia*. *FEMS Microbiol Lett* **151**, 145–153.

Ng, A., Melvin, W. T. & Hobson, P. N. (1994). Identification of anaerobic digester bacteria using a polymerase chain reaction method. *Bioresour Technol* **47**, 73–80.

Noeth, C., Britz, T. J. & Joubert, W. A. (1988). The isolation and characterization of the aerobic endospore-forming bacteria present in the liquid phase of an anaerobic fixed-bed digester, while treating a petrochemical effluent. *Microb Ecol* **16**, 233–240.

Oyaizu, H. & Komagata, K. (1983). Grouping of *Pseudomonas* species on the basis of cellular fatty acid composition and the quinone system with special reference to the existence of 3-hydroxy fatty acids. *J Gen Appl Microbiol* **29**, 17–40.

Palleroni, N. J. & Bradbury, J. F. (1993). *Stenotrophomonas*, a new bacterial genus for *Xanthomonas maltophilia* (Hugh 1980) Swings *et al.* 1983. *Int J Syst Bacteriol* **43**, 606–609.

Sasser, M. (1990). *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*. Technical Note no. 101. Newark, DE: MIDI Inc.

Smibert, R. M. & Krieg, W. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, chapter 25, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Stanier, R. Y., Palleroni, N. J. & Doudoroff, M. (1966). The aerobic pseudomonads: a taxonomic study. *J Gen Microbiol* **43**, 159–271.

Swings, J., De Vos, P., Van den Mooter, M. & De Ley, J. (1983). Transfer of *Pseudomonas maltophilia* Hugh 1981 to the genus *Xanthomonas* as *Xanthomonas maltophilia* (Hugh 1981) comb. nov. *Int J Syst Bacteriol* **33**, 409–413.

Toerien, D. F. & Hattingh, W. H. J. (1969). Anaerobic digestion. I. The microbiology of anaerobic digestion. *Water Res* **3**, 385–416.

Van den Mooter, M. & Swings, J. (1990). Numerical analysis of 295 phenotypic features of 266 *Xanthomonas* strains and related strains and an improved taxonomy of the genus. *Int J Syst Bacteriol* **40**, 348–369.

Van Zyl, E. & Steyn, P. L. (1992). Reinterpretation of the taxonomic position of *Xanthomonas maltophilia* and taxonomic criteria in this genus. Request for an Opinion. *Int J Syst Bacteriol* **42**, 193–198.

Vauterin, L., Hoste, B., Kersters, K. & Swings, J. (1995). Reclassification of *Xanthomonas*. *Int J Syst Bacteriol* **45**, 472–489.

Wallace, W. H., Rice, J. F., White, D. C. & Sayler, G. S. (1994). Distribution of alginate genes in bacterial isolates from corroded metal surfaces. *Microb Ecol* **27**, 213–223.

Wang, R.-F., Pothuluri, J. V., Steele, R. S., Paine, D. D., Assaf, N. A. & Cerniglia, C. E. (1998). Molecular identification of a *Stenotrophomonas* species used in the bioassay for erythromycin in aquaculture samples. *Mol Cell Probes* **12**, 249–254.

Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Wilkinson, K. G., Dixon, K. W., Sivasithamparam, K. & Ghisalberti, E. L. (1994). Effect of IAA on symbiotic germination of an Australian orchid and its production by orchid-associated bacteria. *Plant Soil* **159**, 291–295.

Yang, P., Vauterin, L., Vancanneyt, M., Swings, J. & Kersters, K. (1993). Application of fatty acid methyl esters for the taxonomic analysis of the genus *Xanthomonas*. *Syst Appl Microbiol* **16**, 47–71.