Taxonomic characterization of nine strains isolated from clinical and environmental specimens, and proposal of *Corynebacterium tuberculostearicum* sp. nov.

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Nine unidentified Gram-positive, lipophilic corynebacteria were isolated from clinical and food samples and subjected to a polyphasic taxonomic analysis. The bacteria were distinguished from *Corynebacterium* species with validly published names by biochemical tests, fatty acid content and whole-cell protein analysis. Comparative 16S rRNA gene sequence analysis demonstrated unambiguously that the nine strains were related phylogenetically to the species '*Corynebacterium tuberculostearicum*' and represented a distinct subline within the genus *Corynebacterium*. On the basis of both phenotypic and phylogenetic evidence, the formal description of *Corynebacterium tuberculostearicum* sp. nov. is proposed. The type strain of *C. tuberculostearicum* is Medalle X^T (= LDC-20^T = CIP 107291^T = CCUG 45418^T = ATCC 35529^T).

INTRODUCTION

Corynebacterium species have been detected in various habitats, including dairy products, soil and vegetables (Brennan *et al.*, 2001; Fudou *et al.*, 2002), and in a large variety of hosts such as tortoises, cats (Collins *et al.*, 2001b), dogs (Collins *et al.*, 1999b), seals (Pascual *et al.*, 1998), goats, cows (Fernandez-Garayzabal *et al.*, 1997) and sheep (Collins *et al.*, 2001a). Several species have been implicated in human disease. *Corynebacterium diphtheriae* is the aetiological agent of diphtheria. Other species are recognized as opportunistic pathogens, and have been isolated from diverse clinical specimens, including the genito-urinary tract (Collins *et al.*, 1999a; Funke *et al.*, 1995; Furness *et al.*, 1979), the respiratory tract (Riegel *et al.*, 1995b, 1997b; Zimmermann *et al.*, 1998), blood samples (Riegel *et al.*, 1997a; Funke *et al.*, 1997c) and surgical infections (Esteban *et al.*, 1999; Yassin *et al.*, 2002b). In the

2 years before this paper was written, nine novel species had been described, namely *Corynebacterium capitovis* (Collins *et al.*, 2001a), *Corynebacterium testudinoris* and *Corynebacterium felinum* (Collins *et al.*, 2001b), *Corynebacterium frenayi* (Renaud *et al.*, 2001), *Corynebacterium aurimucosum* (Yassin *et al.*, 2002a), *Corynebacterium mooreparkense* (Brennan *et al.*, 2001), *Corynebacterium appendicis* (Yassin *et al.*, 2002b), *Corynebacterium efficiens* (Fudou *et al.*, 2002) and *Corynebacterium spheniscorum* (Goyache *et al.*, 2003).

Some species of the genus *Corynebacterium* belong to the normal flora of the skin (Funke *et al.*, 1997a; Collins *et al.*, 1988), while others were isolated from specific cutaneous lesions. In leprosy, *Corynebacterium* spp., referred to as leprosy-derived corynebacteria (LDC) (Beaman *et al.*, 1974), were initially reported as a homogeneous and unique group of organisms within the genus *Corynebacterium*, and were later included in the 'corynebacteria pathogenic for humans' (Danhaive *et al.*, 1982). A novel species, '*Corynebacterium tuberculostearicum*', was described as a lipid-requiring LDC, differing from other corynebacteria by the production of tuberculostearic (10-methyl-octadecanoic) acid (Brown *et al.*, 1984), although this name was never validly published. Since this description, other

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences determined in this study are shown in Fig. 2.

members of the genus *Corynebacterium* have been shown to produce tuberculostearic acid (Funke *et al.*, 1998; Yassin *et al.*, 2002a, b).

Identification of lipophilic corynebacteria appears to be problematic with respect to classic bacteriological methods (Riegel *et al.*, 1995a; Watts *et al.*, 2000). The US Centers for Disease Control and Prevention (CDC) separated lipophilic corynebacteria into groups ANF-1, G-1 and G-2 in addition to groups JK (*Corynebacterium jeikeium*) and D-2 (*Corynebacterium urealyticum*) using biochemical tests (Hollis & Weaver, 1981; Riegel *et al.*, 1992, 1993). According to their ability to ferment fructose, bacteria isolated from urethritis samples were separated into ‘*Corynebacterium genitalium*’ pathogenic biotypes and ‘*Corynebacterium pseudogenitalium*’ saprophytic biotypes (Furness *et al.*, 1979). In disagreement with these data, DNA–DNA hybridizations distinguished five genomic groups of lipid-requiring corynebacteria (Riegel *et al.*, 1995a). Genomospecies I included ‘*C. tuberculostearicum*’ LDC8, strain CDC G 5840 (G-2 group), strain CDC F 8156 (G-1 group) and three different ‘*C. pseudogenitalium*’ biotypes. Numerous descriptions of novel lipophilic species of the genus *Corynebacterium* required the use of molecular-based approaches (Riegel *et al.*, 1995a; Neubauer *et al.*, 1991; Funke *et al.*, 1997b; Fernandez-Garayzabal *et al.*, 1997; Yassin *et al.*, 2002b). The implementation of 16S rRNA gene sequencing combined with improved phenotypic approaches (in particular, biochemical characterization using bioMérieux API 50 CH, API ZYM or API Coryne) has provided an accurate system for the identification of novel corynebacteria. Recently, this strategy has led to the incrimination of ‘*C. tuberculostearicum*’ as a causative agent in mastitis (Paviour *et al.*, 2002).

During the course of the characterization of strains deposited in the Collection of the Institut Pasteur (CIP), we identified nine isolates from various clinical and food samples as lipophilic *Corynebacterium* spp. On the basis of

phylogenetic investigations, it is suggested that they be classified as ‘*C. tuberculostearicum*’. Phenotypic comparison of the nine isolates, ‘*C. tuberculostearicum*’ strains CIP 107291^T and CIP 107067 and ‘*C. pseudogenitalium*’ CIP 106714 led us to an emended description of ‘*C. tuberculostearicum*’ and the formal proposal of *Corynebacterium tuberculostearicum* sp. nov.

METHODS

Bacterial strains and culture conditions. The nine unidentified strains were isolated from different sources (Table 1). ‘*C. tuberculostearicum*’ strain CIP 107291^T (=CCUG 45418^T=ATCC 35529^T), also named Medalle X^T or LDC-20^T (Brown *et al.*, 1984; Danhaive *et al.*, 1982), and ‘*C. tuberculostearicum*’ strain CIP 107067 (=CCUG 41662) were originally obtained from the Culture Collection of the University of Göteborg. ‘*C. pseudogenitalium*’ (C-1 type) strain CIP 106714 (=CECT 763=ATCC 33035) (Furness *et al.*, 1979) was obtained from the Spanish Type Culture Collection (CECT). The 12 strains were grown on trypto casein soy agar (Bio-Rad) supplemented with 1 % (v/v) Tween 80 (Riegel *et al.*, 1995a; Watts *et al.*, 2000).

Biochemical characterization. Four commercially available galleries, API Coryne, API ZYM, Api 50 CH and Biotype-100 strips, were used according to the instructions of the manufacturer (bioMérieux) to determine biochemical characteristics, i.e. fermentation profiles, enzymic reactions and assimilation tests. They were inoculated with a bacterial suspension enriched with 1 % (v/v) Tween 80.

PAGE analysis of whole-cell protein. Cellular protein extracts for PAGE analysis were prepared as described previously (Barreau & Hansen, 2000). Densitometric analysis, normalization and comparison of protein patterns were performed using GelCompar version 2.5 (Applied Maths).

Fatty acid analysis and tuberculostearic acid detection. Fatty acid methyl esters and mycolic acid methyl esters were extracted as described previously (Phetsuksiri *et al.*, 1999). Detection of mycolic acids was performed with TLC (Minnikin *et al.*, 1980). Fatty acid methyl esters were analysed by GC on a Shimadzu GC-14A chromatograph using a methyl silicone 5 % phenyl column operating at a temperature of 175 °C for 2 min followed by a programmed increase of 8 °C min⁻¹ to 300 °C. The eluted peak of tuberculostearic

Table 1. ‘*C. tuberculostearicum*’ reference strains and unnamed lipophilic corynebacteria used in this study

Strain	Date	Country (town) of origin	Clinical or environmental source (pathology)
‘ <i>C. tuberculostearicum</i> ’ CIP 107291 ^T	1901	Philippines	Bone marrow (leprosy)
‘ <i>C. tuberculostearicum</i> ’ CIP 107067	1999	Sweden	Contaminant, industrial laboratory
CIP 101775	1985	France (Lille)	Inguinal node (soft chancre)
CIP 102076	1985	France	Unknown
CIP 102124	1986	France (Bordeaux)	Tinned tuna
CIP 102211	1986	France (Royan)	Lymph node
CIP 102346	1986	France (Le Rancy)	Blood
CIP 102590	1986	France (Bordeaux)	Urethra
CIP 102622	1986	France (Paris)	Skin (no leprosy lesion)
CIP 102645	1986	France (Sens)	Peritoneum (peritonitis)
CIP 102857	1987	France (Paris)	Urine
‘ <i>C. pseudogenitalium</i> ’ CIP 106714	1979	Unknown	Healthy urogenital tract

acid was identified by comparing its retention time with the retention time of the tuberculostearic acid standard of *Mycobacterium tuberculosis*.

Genomic DNA extraction. Bacteria were scraped from the agar and resuspended in 200 µl TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 7.4). After the addition of 50 µl lysozyme (5 mg ml⁻¹, pH 7) and 30 µl RNase A from bovine pancreas (10 mg ml⁻¹), the samples were incubated for 30 min at 37 °C. Each lysate was transferred to a 2 ml tube (Sarstedt) containing 20 µl 10% SDS, 0.2 g acid-washed glass beads (212–300 µm; Sigma) and 200 µl phenol/chloroform/isoamyl alcohol (25:24:1 by vol.). The tubes were shaken for two periods of 40 s at a speed of 6 m s⁻¹ in a Savant FastPrep apparatus and then centrifuged at 14 000 r.p.m. for 20 min. The upper phase containing DNA was precipitated with absolute ethanol at -20 °C. After centrifugation, the pellets were washed with 70% ethanol, dried and dissolved in 200 µl TE buffer.

16S rRNA gene amplification. 16S rRNA genes were amplified by PCR using the universal primers A (5'-AGAGTTTGATCCTGGCT-CAG-3', positions 8–27 according to the *Escherichia coli* numbering system; Brosius *et al.*, 1978) and H (5'-AAGGAGGTGATCCAGC-CGCA-3', positions 1541–1522) (Böttger, 1989). Each reaction was performed in a 100 µl volume containing 20 pmol each primer, 2.5 U *Taq* DNA polymerase (Roche Applied Science), 10 µl reaction buffer containing MgCl₂, 0.3 mM each dNTP (Pharmacia Biotech) and 1 µl template DNA. PCR amplification was carried out in a GeneAmp thermal cycler (Applied Biosystems), using the following thermal profile: 94 °C for 4 min, followed by 25 cycles of 94 °C for 1 min, 57 °C for 1 min and 72 °C for 2 min, with a final extension step at 72 °C for 5 min. PCR products were examined using 1% (w/v) agarose gel electrophoresis and ethidium bromide staining. SmartLadder (Eurogentec) was used as a molecular size marker.

16S rRNA gene sequencing. Each PCR product was treated using the ExoSAP-IT exonuclease I and shrimp alkaline phosphatase protocol (USB). PCR products were then sequenced using the *Taq* DyeDeoxy terminator cycle sequencing kit and an ABI PRISM 3700 DNA sequencer according to the instructions of the manufacturer (PE Applied Biosystems). Primers A and H and sequencing primers 5'-CTCCTACGGGAGGCAGCAGT-3' (positions 339–358 according to the *E. coli* numbering system), 5'-ACTGCTGCCTCCGTAGG-AG-3' (positions 358–339), 5'-CGTGCCAGCAGCCGCGTAAT-3' (positions 514–534), 5'-GCATGTGGTTTAATTTCGA-3' (positions 947–964), 5'-TCGAATTAAACCACATGC-3' (positions 964–947) and 5'-AGGGTTGCGCTCGTTCGCGG-3' (positions 1115–1097) were used. The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences determined in this study are shown in Fig. 2.

Computational analysis. The 16S rRNA sequences were compared with known sequences in the GenBank/EMBL/DBJ databases by using the BLASTN 2.2.4 algorithm (Altschul *et al.*, 1997). Sequences were then aligned using the CLUSTAL V algorithm (Higgins, 1994) with default gap penalties. The initial alignment was further refined by eye, introducing gaps to improve the overall alignment, using the alignment editor of the PAUP* version 4.0 software package (Swofford, 1998). Sequence-distance matrices were established in pairwise comparisons by applying the algorithm of Tamura & Nei (1993). Phylogenetic trees were constructed by the neighbour-joining method (Saitou & Nei, 1987) using the PHYLIP version 3.5 software package (Felsenstein, 1993). A maximum-likelihood analysis was performed using the same model and an estimated rate gamma shape of 0.7484 with PAUP* 4.0 version (Felsenstein, 1981). Maximum-parsimony trees were obtained using 100 random-addition heuristic search replicates and the tree bisection–reconnection

branch-swapping option. Statistical significance was evaluated by bootstrap analysis (Felsenstein, 1985), with 100 iterations of bootstrap samplings.

RESULTS AND DISCUSSION

Phenotypic characterization of strains

The nine unidentified strains were non-motile, non-spore-forming, pleomorphic, Gram-positive rods, frequently swollen at one or both ends, and aerobic to facultatively anaerobic. On trypto casein soy agar supplemented with 1% (v/v) Tween 80, colonies were whitish and glistening with entire edges (1 mm diameter after 24–48 h at 37 °C). All isolates were oxidase-negative, catalase-positive and urease-negative. Most had enzymic activities similar to those of '*C. tuberculostearicum*' CIP 107291^T, CIP 107067 and '*C. pseudogenitalium*' CIP 106714 in the API ZYM system. Reactions for esterase (C4) and naphthol-AS-BI-phosphohydrolase were positive for all strains. No activity was detected for α-galactosidase, β-galactosidase, α-fucosidase, lipase (C14), trypsin or α-chymotrypsin. Results for nitrate reductase, esterase lipase (C8), leucine arylamidase and alkaline phosphatase production are shown in Table 2. With API 50 CH, acid was produced from galactose, glucose, glycerol, fructose, mannose, ribose and 5-ketogluconate but not from mannitol, glycogen, starch, lactose or xylose. Sucrose and maltose were not fermented by any of the strains, even after 3 days incubation (Table 2). Biotype-100 strips showed that the substrates D-glucose, sucrose, D-ribose, glycerol, L-malate, 2-ketogluconate, succinate, fumarate, L-aspartate,

Table 2. Differential characteristics of *C. tuberculostearicum* and its nearest phylogenetic relatives

Species/strains: 1, *C. accolens* CIP 104783^T; 2, '*C. fastidiosum*' CIP 103808; 3, *C. macginleyi* CIP 104099^T; 4, '*C. segmentosum*' CIP 107068; 5, *C. tuberculostearicum* (*n*=11); 6, '*C. pseudogenitalium*' CIP 106714. Values for *C. tuberculostearicum* are percentages of strains giving positive results.

Characteristic	1	2	3	4	5	6
Production of:						
Nitrate reductase	+	+	+	+	27	–
Pyrazinamidase	–	–	–	–	100	+
Alkaline phosphatase	–	+	+	+	91	+
Leucine arylamidase	+	+	–	–	91	+
α-Glucosidase	+	–	–	+	0	–
Esterase lipase	+	+	+	+	91	+
Fermentation of:						
Sucrose	–	+	+	+	82	+
Maltose	+	+	–	–	91	–
Glycerol	+	–	+	–	100	+
Galactose	–	–	+	–	100	+

L-glutamate, L-proline and L-serine were always used as sole carbon sources. L-Alanine was assimilated by all strains except '*C. tuberculostearicum*' CIP 107067. 2-Ketogluconate was assimilated by all strains except '*C. pseudogenitalium*' CIP 106714. The API Coryne system generated the numerical code 6100324 for '*C. tuberculostearicum*' strains CIP 107291^T and CIP 102590, 6100325 for strains CIP 102211, CIP 102346, CIP 101775, '*C. tuberculostearicum*' CIP 107067 and '*C. pseudogenitalium*' CIP 106714, 7100325 for strains CIP 102124, CIP 102857 and CIP 102076, 6100305 for strain CIP 102622 and 2100325 for strain CIP 102645. These codes corresponded to *Corynebacterium* CDC group G in the API Coryne profile index. GC analyses of non-hydroxylated fatty acid methyl esters showed that C_{18:0}, C_{18:1cis9} and C_{18:2} were the predominant cellular fatty acids in the nine strains, '*C. tuberculostearicum*' CIP 107291^T and CIP 107067, in agreement with a previous study (Bernard *et al.*, 1991). Small amounts of tuberculostearic acid (0.83–4.42%) and mycolic acids were also detected in the strains. All these strains clustered together in SDS-PAGE analysis and formed a distinct branch with a correlation level of more than 72% (Fig. 1). These chemotaxonomic findings demonstrated that the nine strains, '*C. tuberculostearicum*' strains CIP 107291^T and CIP 107067 and '*C. pseudogenitalium*' CIP 106714 represented a homogeneous group.

Phylogenetic analysis

To ascertain the phylogenetic positions of the isolates, complete 16S rRNA gene sequences (1512 nucleotides) of the nine unidentified strains, '*C. tuberculostearicum*' CIP 107291^T and CIP 107067 and '*C. pseudogenitalium*' CIP

106714 were determined and subjected to a comparative analysis. Sequence searches of the GenBank/EMBL/DDBJ databases revealed that the nine unidentified strains were closely related to species of the genus *Corynebacterium*. The 16S rRNA gene sequences of the nine strains displayed high levels of similarity (89.8–98.9%) to those of previously described members of the genus *Corynebacterium*. A preliminary phylogenetic analysis based on *Corynebacterium* 16S rRNA gene sequences deposited in international databases showed that the nine sequences clustered with '*C. tuberculostearicum*' ATCC 35692^T (accession no. X84247) and '*C. pseudogenitalium*' ATCC 33035 (U87822), confirming that the isolates were members of the genus *Corynebacterium* (data not shown). A neighbour-joining phylogenetic tree depicting the relationships between the strains and the nearest *Corynebacterium* species is shown in Fig. 2. The 16S rRNA gene sequences of the isolates grouped with those of '*C. pseudogenitalium*' CIP 106714 and '*C. tuberculostearicum*' strains CIP 107291^T and CIP 107067. Comparison of sequences from this monophyletic group revealed 0–8 nucleotide differences (corresponding to 100–99.2% similarity). The high levels of 16S rRNA gene sequence similarity suggested that the nine strains, '*C. tuberculostearicum*' strains CIP 107291^T and CIP 107067 and '*C. pseudogenitalium*' CIP 106714 belonged to the same species (Drancourt *et al.*, 2000).

Differential properties

The monophyletic group of '*C. tuberculostearicum*' and related strains formed a distinct lineage within a small

Fig. 1. Similarity dendrogram based on whole-cell protein patterns of the nine unidentified strains, '*C. tuberculostearicum*' and related strains. For convenience, levels of correlation are expressed as percentages of similarity.

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences showing the relationships between the nine unidentified strains and '*C. tuberculostearicum*' and some other related taxa. Significant bootstrap values (%) are given at branching points. Bar, 0.005 substitutions per site.

phylogenetic group including *Corynebacterium accolens*, *Corynebacterium macginleyi*, '*Corynebacterium segmentosum*' and '*Corynebacterium fastidiosum*'. Sequence similarities between '*C. tuberculostearicum*' taxa and *C. accolens*, *C. macginleyi*, '*C. segmentosum*' and '*C. fastidiosum*' ranged from 97.54 to 98.57 %. These similarity values, alone, are too high (above 97 %) to distinguish '*C. tuberculostearicum*' formally from closely related species (Stackebrandt & Goebel, 1994). Previous studies have shown that numerous species of the genus *Corynebacterium* that exhibited only limited 16S rRNA divergence presented sufficient distinctive characters, fully justifying their classification in separate species (Pascual *et al.*, 1995). This was the case for *Corynebacterium ulcerans*, *C. diphtheriae* and *Corynebacterium pseudotuberculosis*, which share more than 98 % 16S rRNA similarity. Similarly, *Corynebacterium propinquum* and *Corynebacterium pseudodiphtheriticum* share more than 99 % 16S rRNA similarity (Pascual *et al.*, 1995; Ruimy *et al.*, 1995). Thus, the 97 % limit is not decisive for separating species of the genus *Corynebacterium*: further characteristics are required. Biochemical features such as production of alkaline phosphatase, leucine arylamidase, nitrate reductase, esterase lipase, pyrazinamidase and α -glucosidase or fermentation of glycerol and galactose confirmed that the 12 strains were distinct from *C. accolens* CIP 104783^T, *C. macginleyi* CIP 104099^T, '*C. segmentosum*' CIP 107068 and '*C. fastidiosum*' CIP 103808 (Table 2). The type strain of *C. accolens* produced acid from maltose, as described previously (Neubauer *et al.*, 1991). However, another study showed that only 7 % of strains of *C. accolens* studied fermented maltose (Riegel *et al.*, 1995a). Thus, the fermentation of maltose becomes an unreliable characteristic, as reported previously (Funke *et al.*, 1997a). We also noted that acid was produced from glycogen by *C. accolens* CIP 104783^T with API 50 CH but not with API Coryne, while the 11 strains of '*C. tuberculostearicum*', *C. genitalium* CIP 106714, *C. macginleyi* CIP 104099^T, '*C. segmentosum*' CIP 107068 and '*C. fastidiosum*' CIP 103808 did not ferment glycogen with either API 50 CH or API Coryne. SDS-PAGE patterns of *C. accolens* CIP 104783^T, *C. macginleyi* CIP 104099^T, '*C. segmentosum*' CIP 107068 and '*C. fastidiosum*' CIP 103808 did not display a close affinity with those of '*C. tuberculostearicum*'. In addition, a previous DNA hybridization study showed clearly that *C. accolens*, *C. macginleyi* and '*C. tuberculostearicum*' (strain LDC 8) belong to separate genomic species (Riegel *et al.*, 1995a). Recently, a lipophilic species, *C. appendicis*, isolated from humans, has been described (Yassin *et al.*, 2002b). This novel species can be differentiated from '*C. tuberculostearicum*' by its ability to hydrolyse urea and by its position in the 16S rRNA phylogenetic tree (Fig. 2).

Consequently, we feel that, on the basis of phenotypic data, whole-cell protein profiles and 16S rRNA sequence analysis, it is reasonable to assign the nine *Corynebacterium* strains CIP 101775, CIP 102076, CIP 102124, CIP 102211, CIP 102346, CIP 102590, CIP 102622, CIP 102645, CIP 102857 and '*C. pseudogenitalium*' CIP 106714 to the species

Corynebacterium tuberculostearicum. An emended description of this species is presented below.

Description of *Corynebacterium tuberculostearicum* sp. nov.

Corynebacterium tuberculostearicum (tu.ber.cu.lo.stea'ri.cum. N.L. neut. adj. *tuberculostearicum* of tuberculostearic acid, which is contained in the cells).

Cells are pleomorphic, Gram-positive rods, non-motile and non-spore-forming, developing coccoid forms in stationary cultures. Colonies on trypto casein soy agar supplemented with Tween 80 are circular, convex, glistening and 1 mm in diameter. Aerobic to facultatively anaerobic. Lipophilic. Oxidase-negative and catalase-positive. *Corynebacterium* mycolic acids are present and the fatty acid profiles contain tuberculostearic acid. Acid is produced from galactose, glucose, glycerol, fructose, mannose, ribose and 5-ketogluconate but not from mannitol, glycogen, starch, sorbitol, lactose, inulin or xylose. Production of acid from trehalose, maltose, gluconate, sucrose and *N*-acetylglucosamine is variable. Urea, aesculin and gelatin are not hydrolysed. Presence of nitrate reductase is variable. DNase is absent. Esterase (C4) and naphthol-AS-BI-phosphohydrolase activities are detected. Presence of esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and alkaline phosphatase is variable. No activity is detected for α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, α -fucosidase, lipase (C14), trypsin or α -chymotrypsin. The following substrates are utilized in 2 or 4 days: D-glucose, sucrose, D-ribose, glycerol, L-malate, 2-ketogluconate, succinate, fumarate, L-aspartate, L-glutamate, L-proline and L-serine. The following substrates are not utilized in 6 days: caprate, citrate and DL-glycerate.

The type strain, Medalle X^T (= LDC-20^T = CIP 107291^T = CCUG 45418^T = ATCC 35529^T), was isolated from a case of lepromatous leprosy in the Philippines (Brown *et al.*, 1984). The type strain has all the properties given for the species. In addition, it assimilates D-mannose.

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