Corynebacterium pyruviciproducens sp. nov., a pyruvic acid producer

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A coryneform strain, 06-17730T (=WAL 19168T), derived from a groin abscess sample was characterized using phenotypic and molecular taxonomic methods. Comparative analyses revealed more than 3% divergence of the 16S rRNA gene sequence and about 10% divergence of the partial rpoB gene sequence from the type strain of Corynebacterium glucuronolyticum. The strain could also be differentiated from C. glucuronolyticum by a set of phenotypic properties. A DNA–DNA relatedness study between strain WAL 19168T and C. glucuronolyticum CCUG 35055T showed a relatedness value of 13.3% (13.7% on repeat analysis). The genotypic and phenotypic data show that the strain merits classification within a novel species of Corynebacterium. We propose the name Corynebacterium pyruviciproducens sp. nov. for the novel species. The type strain is 06-17730T (=WAL 19168T =CCUG 57046T =ATCC BAA-1742T).

Bacteria of the genus Corynebacterium have been recovered extensively from mammals and the environment (Feurer et al., 2004; Hommez et al., 1999). Except for Corynebacterium diphtheriae, the aetiological agent of diphtheria, most species of the genus are regarded as opportunistic pathogens (Paviour et al., 2002). Compared with those bacteria possessing proof of clinical pathogenicity, less emphasis has been put on Corynebacterium. Recently, however, a report demonstrated that Corynebacterium was the most prevalent bacterial genus associated with chronic wound infections in diabetics (possibly skin flora) (Dowd et al., 2008). In our investigation of the bacteriology of acute wound infections (Finegold et al., 2008), a novel aerobic, rod-shaped, Gram-positive corynebacterium was found. It is similar to Corynebacterium glucuronolyticum (Devriese et al., 2000) by phenotypic and genotypic identification. The aim of this polyphasic study was to describe the isolation and characterization of this novel organism.

A groin abscess specimen was collected at Olive View-UCLA Medical Center in 2006, and studied in the Wadsworth Anaerobic Laboratory (WAL). One isolate derived from this specimen, WAL 19168T, was analysed by phenotypic and genotypic characterization methods. Optimal growth conditions were investigated by cultivating strain WAL 19168T aerobically and anaerobically on tryptase soy agar with 5% sheep blood (TSA-II; Becton Dickinson Microbiology Systems) at 20, 37 and 42°C. Lipid requirement was determined by comparison between the culture in simple brain heart infusion (BHI) broth (MP Biomedicals) and in the same medium supplemented with 1% Tween 80 after 72 h at 37°C (Riegel et al., 1994). Further confirmation of lipid stimulation of growth was observed by the 'response to serum' test (Coyle & Lipsky, 1990).

Strain WAL 19168T and reference strains were characterized by routine tests (Devriese et al., 2000; Wattiau et al.,...
including Gram staining, API Coryne and API ZYM strips (bioMérieux) and susceptibility to the vibriostatic agent O/129 (150 μg; Oxoid) (Bernard et al., 2002). The Christie–Atkins–Munch-Petersen (CAMP) reaction and fermentative test from fructose were confirmed by conventional methods (Riegel et al., 1994, 1995). The reference strains included in the above procedures were \( C. \) glucuronolyticum CCUG 35055\(^T\), \( C. \) urealyticum CCUG 18158\(^T\), \( C. \) accolens CCUG 28779\(^T\) and \( C. \) jeikeium CCUG 27192\(^T\), received from the Culture Collection of the University of Göteborg. Additionally for strain WAL 19168\(^T\), susceptibility to antibiotics (0.016–256 μg ampicillin ml\(^{-1}\), 0.002–32 μg ceftriaxone ml\(^{-1}\), 0.016–256 μg clindamycin ml\(^{-1}\) and 0.016–256 μg erythromycin ml\(^{-1}\) was studied by the Etest system (AB Biodisk) with MIC value confirmation according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. \( \beta \)-Lactamase activity was assayed by the nitrocefin disc test (Biotech Diagnostics). All strains were cultured under the same conditions and tests were performed in duplicate with 24–48 h pure cultures according to the manufacturers’ instructions, unless otherwise noted.

For cellular fatty acid (CFA) and metabolic end product (ME) (short-chain volatile and non-volatile fatty acids) analyses, the strains were grown in BHI broth supplemented with 1% glucose and 5% rabbit serum (MP Biomedicals). CFAs of the strains were detected with a Hewlett Packard 5890 series II gas chromatograph, while MEs were tested by GLC equipped with a flame-ionization detector (Finegold et al., 2004). Short-chain mycolic acids were investigated by a reversed-phase HPLC method (De Briel et al., 1992). Microbial Identification System software (MIDI, Inc.) was applied to generate and analyse the profiles of MEs and CFAs, as described previously (Song et al., 2006).

Genomic DNA of strain WAL 19168\(^T\) was extracted and purified using a QIamp DNA Mini kit (Qiagen). PCRs were used for the amplification of the 16S rRNA gene (Song et al., 2003) and the partial \( rpoB \) gene (with primers C2625F and C3130R) (Khamis et al., 2004). The products were sequenced directly on a 3130 Genetic Analyzer (Applied Biosystems). The sequences obtained were compared with sequences in the GenBank database by using BLAST software (Brosius et al., 1978); similarity searches were performed with CLUSTAL W and distance matrices were created with respect to 1409 bp of the 16S rRNA gene sequence and 508 bp of the partial \( rpoB \) gene sequence. The neighbour-joining (Saitou & Nei, 1987) and UPGMA (Sneath & Sokal, 1973) methods were used to construct phylogenetic trees. The DNA G+C content of the novel organism was determined by HPLC (Mesbah et al., 1989). A DNA–DNA reassociation test between strain WAL 19168\(^T\) and \( C. \) glucuronolyticum CCUG 35055\(^T\) was done by using photobiotin-labelled probes in microplate wells (Ezaki et al., 1989).

Strain WAL 19168\(^T\) was recovered from the groin abscess with a count of \( 10^8 \) c.f.u. ml\(^{-1}\), accompanied by strains of \( A. \) radingae (10\(^6\) c.f.u. ml\(^{-1}\)), \( P. \) avidum (10\(^8\) c.f.u. ml\(^{-1}\)) and \( P. \) acnes (10\(^7\) c.f.u. ml\(^{-1}\)). As with \( C. \) glucuronolyticum, strain WAL 19168\(^T\) was able to grow on TSA-II plates at 37 and 42 °C aerobically as well as anaerobically after 2 days of incubation, but not at 20 °C. In addition, growth was visible in BHI broth supplemented with 1% Tween 80 but not in non-supplemented infusion, better under aerobic conditions than in an anaerobic jar, and more abundant at 37 than at 42 °C. The most profuse growth was exhibited in serum-supplemented liquid medium. In contrast to the opaque and whitish-pale colonies (1–1.5 mm in diameter) of \( C. \) glucuronolyticum, the colonies of strain WAL 19168\(^T\) were small (0.3–0.5 mm in diameter), circular, entire, convex and translucent (Fig. 1). Blood plates on which \( C. \) glucuronolyticum CCUG 35055\(^T\) was grown turned tan after approximately 24 h of incubation, while plates on which strain WAL 19168\(^T\) was inoculated did not change colour until 72 h later. Microscopy revealed that the cells of strain WAL 19168\(^T\) were Gram-positive, non-motile and rod-shaped, with occasional swellings.

According to tests on the API Coryne strip (code 2200725), the novel isolate was pyrazinamidase- and catalase-positive, weakly positive for pyrrolidonyl arylamidase and negative for gelatin hydrolysis, urease and reduction of nitrate. It could use D-ribose slightly and was able to ferment D-xylene, D-glucose, maltose and sucrose, but was unable to utilize D-mannitol, lactose (bovine origin) and glycerogen. The API ZYM strip revealed that strain WAL 19168\(^T\) could produce esterase (C4), esterase lipase (C8), leucine arylamidase and a little naphthol-AS-BI-phosphohydrolase. The reactions for acid phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, z-chymotrypsin, z-galactosidase, z-fucosidase and z-mannosidase were negative. API Coryne and API ZYM strips both gave positive reactions for \( \beta \)-glucuronidase and negative tests for z-glucosidase, \( \beta \)-galactosidase, N-acetyl-\( \beta \)-glucosamini-

**Fig. 1.** Colonial appearance of \( C. \) glucuronolyticum CCUG 35055\(^T\) (a) and strain WAL 19168\(^T\) (b) on TSA-II plates after 2 days of incubation. Note the differences in the size of colonies and the colour of the plates.
tuberculostearic acid (TBSA; 10-methyl C18 : 0) was found; the amount of propionic acid; the major MEs were acetic acid and the type strains of related species.

WAL 19168T could utilize glucose and produced a small amount of propionic acid; the major MEs were acetic acid (volatile fatty acid; 817 μg ml⁻¹) and pyruvic acid (non-volatile fatty acid; 817 μg ml⁻¹).

The main CFA detected in strain WAL 19168T was C₁₈₋₁₀₉₆, comprising 28% of the total fatty acids. No tuberculostearic acid (TBSA; 10-methyl C₁₈₋₀) was found; other CFAs detected at 10% or more of the total fatty acids were C₁₆₋₀ and summed feature 8 (C₁₇₋₁₀₈c and/or C₁₇₋₁₂).

Short-chain mycolic acids (C₂₂–C₃₆) were present. Strain WAL 19168T showed a reassociation value of 13.3% (13.7% on repeat analysis), confirming that these are members of distinct species. Phylogenetic trees derived from alignments of the 16S rRNA and _rpoB_ gene sequences are shown in Fig. 2 and Supplementary Fig. S1 (available in IJSEM Online).

The novel coryneform currently described is evidently lipophilic, based on our research on its growth conditions. We therefore chose the type strains of several medically significant lipophilic _Corynebacterium_ species, i.e. _C. jeikeium_, _C. urealyticum_ and _C. accolens_ as reference strains in addition to genetically related species. They are the members of coryneform CDC groups JK, D and G, respectively (Coyle & Lipsky, 1990; Pitcher _et al._, 1992; Neubauer _et al._, 1991). In our research, strain WAL 19168T possessed a strongly positive catalase reaction, in common with other members of the genus _Corynebacterium_. However, it is known that the identification of members of the genus _Corynebacterium_ is difficult in clinical and reference laboratories (Clarridge, 1986; Sabbe _et al._, 1999). The API Coryne strip of strain WAL 19168T in our study gave the result ‘_Corynebacterium glucuronolyticum_ with 99.9% ID’. Therefore, more supplementary tests should be performed to help classification, such as the CAMP test, fermentative activity from fructose and production of cystine arylamidase and acid phosphatase. In addition, the novel organism can be differentiated from important lipophilic strains by β-glucuronidase production and _D_-xylose fermentation tests. Regarding the CFAs of the novel strain, we did not find significant differences from the profiles of other _Corynebacterium_ species (Van den Velde _et al._, 2006). It should be noted that this strain was able to metabolize glucose and produce pyruvic acid at high concentrations, which can be useful in identifying the novel organism, although they are not unique characteristics within the genus _Corynebacterium_. Pyruvic acid is the major metabolic intermediate during the metabolic cycle of corynebacteria and can be produced by several species (Rollin _et al._, 1995), but most of these are mutant strains developed for industrial purposes. So far, no _Corynebacterium_ species recovered from human sources has been reported to have this ability.

Comparisons based on the GenBank database showed that the closest match to strain WAL 19168T was _C. glucuronolyticum_ CCUG 35055ᵀ, with 96.8% sequence similarity for the 16S rRNA gene sequence and 90.6% sequence similarity for the partial _rpoB_ gene sequence. The DNA G+C content of strain WAL 19168T is 62 mol%. This is within the range described for the genus _Corynebacterium_ (51–70 mol%; Cerdeno-Tarraga _et al._, 2003). A DNA–DNA reassociation study between strain WAL 19168T and _C. glucuronolyticum_ CCUG 35055ᵀ showed a reassociation value of 13.3% (13.7% on repeat analysis), confirming that they are members of distinct species. Phylogenetic trees derived from alignments of the 16S rRNA and _rpoB_ gene sequences are shown in Fig. 2 and Supplementary Fig. S1 (available in IJSEM Online).

Table 1. Biochemical characteristics of strain WAL 19168ᵀ and the type strains of related species

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Strains: 1, _C. pyruviciproducens_ sp. nov. WAL 19168ᵀ; 2, _C. glucuronolyticum_ CCUG 35055ᵀ; 3, _C. urealyticum_ CCUG 18158ᵀ; 4, _C. accolens_ CCUG 28779ᵀ; 5, _C. jeikeium_ CCUG 27192ᵀ. +, Positive; −, negative; w, weakly positive reaction. Data were obtained in this study.
Fig. 2. Neighbour-joining phylogenetic trees showing the position of strain WAL 19168\textsuperscript{T} (=06-1773\textsuperscript{0}), based on comparisons of 16S rRNA gene sequences of 1409 bp (a) and partial rpoB gene sequences of 508 bp (b). Bootstrap percentages (based on 1000 replications) are shown at branching points. GenBank accession numbers for each sequence are given in parentheses. Bars, 0.2 (a) and 1 (b) substitutions per site.
similarity of the single-copy rpoB gene can be an efficient supplement to these traditional taxonomic methods, since cases of distinct species exhibiting 16S rRNA gene sequence similarity >99 % are often found (Adékambi et al., 2008). Furthermore, an rpoB gene sequence similarity of >97.7 % has already been confirmed to be useful in delineating species. In our study, 16S rRNA gene sequence comparison and a similarity value of 90.6 % for the partial rpoB gene sequence indicated that the unknown bacterium isolated from a clinical wound specimen may well represent a new genospecies of Corynebacterium. All of the phylogenetic analyses demonstrate that the novel strain appears to represent a new subline of the genus Corynebacterium, for which the name Corynebacterium pyruviciproducens sp. nov. is proposed.

Description of Corynebacterium pyruviciproducens sp. nov.

Corynebacterium pyruviciproducens (py.ru.vi’ci.pro.du’cens. N.L. n. acidum pyruvicum pyruvic acid; L. part. adj. producens producing; N.L. part. adj. pyruviciproducens pyruvic acid-producing).

Gram-positive, non-spor-forming rods; non-motile. Lipophilic. Can grow aerobically or facultatively anaerobically at 37 and 42 °C. Optimum growth at 37 °C under aerobic conditions. Colonies are small (0.3–0.5 mm in diameter), circular, entire, convex and translucent after 2 days of incubation on blood agar. Strongly catalase-positive, but urease-negative. Cannot reduce nitrites and CAMP test is negative. Acid is produced from D-ribose, D-xylene, D-glucose, maltose and sucrose, but not from fructose, D-mannitol, lactose (bovine origin) or glycogen. Cells produce esterase (C4), esterase lipase (C8), leucine arylamidase, pyrazinamidase, a small amount of naphthaldehyde-BI-phosphohydrolase and pyrrolidonyl arylamidase, but no acid phosphatase, β-galactosidase, α-glucosidase, N-acetyl-β-glucosaminidase, lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, α-galactosidase, α-fucosidase, alkaline phosphatase, α-mannosidase or β-glucosidase. Trypsin and gelatin are not hydrolysed. β-Lactamase-negative. Susceptible to the vibriostatic agent O/129 and highly sensitive to ampicillin, ceftriaxone, clindamycin and erythromycin. The major cellular fatty acids are C18:1ω9c, C16:0 and summed feature 8 (C17:1ω8c and/or C17:0ω6c3); no TBSA is produced. Short-chain mycolic acids (C22–C36) are present. Major MEs from glucose are acetic acid and pyruvic acid.

The type strain is 06-1773Q^T (=WAL 19168^T =CCUG 57046^T =ATCC BAA-1742^T), isolated from a groin abscess specimen taken at Olive View-UCLA Medical Center in 2006. The DNA G+C content of the type strain is 62 mol%.

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References


