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Aureobacterium resistens sp. nov., exhibiting vancomycin resistance and teicoplanin susceptibility

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Abstract

Two similar strains of a coryneform bacterium were isolated from human clinical material. Both strains were resistant to vancomycin but susceptible to teicoplanin. Detailed biochemical, chemotaxonomical, and molecular genetic investigations revealed that both isolates were members of a hitherto undescribed species of the genus *Aureobacterium*. The name *Aureobacterium resistens* sp. nov. is proposed for the new bacterium and the type strain is CCUG 38312.

Keywords: *Aureobacterium resistens* sp. nov.; *Aureobacterium* taxonomy; Vancomycin resistance

1. Introduction

In recent years, clinical microbiologists have become more aware both of the clinical significance as well as of the enormous diversity within the group of coryneform bacteria [1]. Strains belonging to genera which were previously only relevant to environmental microbiologists were also shown to occur in human clinical specimens [1]. One of these medically relevant genera is the genus *Aureobacterium*, members of which had been for many years mistakingly included in the '*Corynebacterium aquaticum*' group of bacteria [2]. In 1996, Saweljew et al. reported a fatal systemic infection with an *Aureobacterium* sp. 'Mainz' in an immunocompetent patient [3]. Nolte et al. [4] presented the first report from the U.S. of a lethal bacteremia due to a vancomycin-resistant, teicoplanin-susceptible *Aureobacterium* species. Independently, a second *Aureobacterium* strain with the same antimicrobial susceptibility pattern had been isolated in Europe. The present report deals with the comprehensive characterization, both phenotypic and phylogenetic, of these latter two unusual strains of coryneform bacteria, resulting in the description of a new *Aureobacterium*, *Aureobacterium resistens* sp. nov.

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2. Materials and methods

2.1. Microorganisms and cultivation

Aureobacterium sp. strain DMMZ 1589 (Culture Collection University of Göteborg, Sweden [CCUG] 38311) was isolated in 1995 in Atlanta from a blood culture of a 39 year-old male with acute myelogenous leukemia [4]. *Aureobacterium* sp. strain DMMZ 1710 (CCUG 38312) had been isolated in 1995 in Zürich from a corneal ulcer of a 14 year-old female wearing contact lenses. Both strains were subcultured on Columbia agar supplemented with 5% sheep blood (SBA) for 24 h in a 5% CO_2-enriched atmosphere and from these plates cells were taken for further testing.

2.2. Phenotypical analysis

The biochemical profiles of strains DMMZ 1589 and DMMZ 1710 were determined as described previously [5]. Hydrolysis of tyrosine, casein, and starch were tested according to Nash and Krenz [6]. Carbohydrate utilization patterns were tested in the API 50CH gallery (bioMérieux, Marcy l'Etoile, France) [7]. Chemotaxonomic investigations included determination of cellular fatty acids (CFAs) and detailed peptidoglycan structural analysis [2]. The antimicrobial susceptibility patterns of both strains were analyzed with the MCN system (Merlin Diagnostics, Bornheim, Germany) [8] and minimal inhibitory concentrations (MICs in μg/ml) for vancomycin and teicoplanin were also independently determined on Mueller-Hinton agar plates supplemented with 5% sheep blood applying E-test strips (AB Biodisk, Solna, Sweden).

2.3. Phylogenetic analysis

Determination of the 16S rRNA gene sequence of strain DMMZ 1710 (EMBL accession number Y14699) and DMMZ 1589 was by PCR-mediated amplification of the gene followed by cycle sequencing of the PCR product, and electrophoretic analysis using an Applied Biosystems 373A automatic sequencer (Foster City, USA) as described previously [9]. Phylogenetic analysis including sequence alignment, calculation of percentage sequence similarity, construction of a phylogenetic tree, and an assessment of the tree topology by bootstrap analysis has also been outlined previously [9].

3. Results and discussion

Colonies of both *Aureobacterium* strains DMMZ 1589 and DMMZ 1710 were up to 1.5 mm in diameter after 24 h incubation, glistening, convex, of creamy consistency, and started to develop a bright yellow pigment. Gram stains showed short (i.e. less than 2 μm in length) and slim Gram-positive rods which were arranged as single cells or in clusters.

The basic biochemical screening reactions [1] were as follows: catalase positive; non-motile; oxidative metabolism; nitrate reduction negative; urea hydrolysis negative; esculin hydrolysis positive; acid production from glucose, maltose, and sucrose but not from mannitol, and xylose; CAMP reaction (see [1] for test performance) negative. In addition, DNase activity was observed after 2 days, tyrosine hydrolysis also became positive after 2 days whereas hydrolysis of casein became positive after 10 days only, and starch hydrolysis was negative. When applying the commercial API Coryne system (bioMérieux) [10] we observed the numerical code 2472325 (a seven digit code corresponding to 21 biochemical reactions) for both strains.

Most interestingly, both strains were found to have a vancomycin MIC of 64 indicating resistance according to NCCLS guidelines but a teicoplanin MIC of 2 indicating susceptibility [11]. Both results were confirmed by the E-test showing a vancomycin MIC of 48 and of 1.5 for teicoplanin. Resistance of this type has not been reported before for any other coryneform bacterium. In Gram-positive cocci, *Enterococcus* spp. may harbour the *vanB* gene showing a similar glycopeptide resistance pattern [12]. However, the *vanB* gene could not be detected in strain DMMZ 1589 [4].

Chemotaxonomic investigations revealed C15:0*ai* (20–23% of total CFAs), C16:0*i* (19–21%), and C17:0*ai* (47–52%) as the predominant CFAs. Smaller amounts of C15:0 (1–2%), C16:0 (3–4%), C17:0*i* (2%), and C18:0*i* (1%) were also detected. Ornithine was found to be the diamino acid of the cell wall peptidoglycan, and its acyl type was of the glycolyl

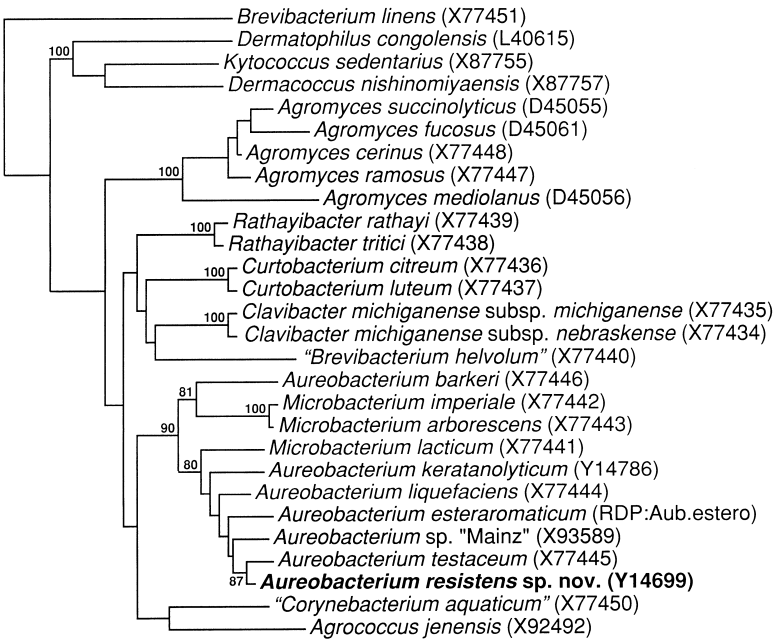

Fig. 1. Unrooted tree showing the phylogenetic position of *A. resistens* within other high G+C content actinomycetes. The tree constructed using the neighbor-joining method was based on a comparison of approximately 1330 nucleotides of 16S rRNA genes. Bootstrap values, expressed as a percentage of 250 replications, are given at the branching points. Numbers in brackets are EMBL/GenBank accession numbers.

type. All these findings were compatible only with an assignment of the two strains to the genus *Aureobacterium* [13]. Detailed peptidoglycan analysis revealed that apart from D-ornithine huge amounts of L-ornithine were present and that both strains belonged to the peptidoglycan type B2α ([L-ornithine]D-glutamic acid-D-ornithine). Within the genus *Aureobacterium*, *A. keratanolyticum* is presently the only species exhibiting this unusual peptidoglycan type [14]. However, our two strains were different from the only one *A. keratanolyticum* strain described in the literature [14] by their ability to hydrolyse esculin and their acid production from glucose, maltose, and sucrose. These data clearly indicated that strains DMMZ 1589 and DMMZ 1710 represent a new *Aureobacterium* species.

In order to investigate the phylogenetic placement of the two clinical strains the genes encoding their 16S rRNAs were amplified by PCR and subjected to sequence analysis. The almost complete 16S rRNA gene sequences (approximately 1420 bp) of both strains were determined and found to be identical (100% sequence similarity), thereby demonstrating that both strains belonged to the same species. Pairwise and treeing analyses revealed *A. testaceum* as the closest (98.7% sequence similarity) phylogenetic neighbour of the clinical strains (Fig. 1). *A. keratanolyticum*, despite exhibiting a similar peptidoglycan type to that of the human isolates, was somewhat more distantly related (98.2% sequence similarity). Considering the high 16S rDNA homologies ($>97.6\%$) within the genus *Aureobacterium* [15,16] it is evident that the two strains represent a new *Aureobacterium* species for which the designation *Aureobacterium resistens* is proposed. The precise molecular mechanism conferring vancomycin resistance while the strains remained susceptible to teicoplanin is currently under investigation.

3.1. Description of *Aureobacterium resistens* sp. nov.

Aureobacterium resistens (re'sis.tens. L. pres. part. *resistens*, being resistant, referring to the vancomycin resistance which is very unusual for coryneform bac-

teria) cells are Gram-positive, asporogenous, small (1 to 2 μm in length) and slim rods which are nonmotile. Colonies are up to 1.5 mm in diameter after 24 h incubation, glistening, convex, of creamy consistency, and exhibit a yellow pigment. Catalase positive. Acid is produced oxidatively from glucose, maltose, and sucrose but not from mannitol and xylose. The following carbohydrates are utilized: glycerol, ribose, galactose, D-fructose, D-mannose, rhamnose, *N*-acetyl-glucosamine, amygdaline, salicine, cellobiose, lactose, trehalose, xylitol, D-turanose, D-lyxose, gluconate, 2-keto-gluconate, and 5-keto-gluconate. The following carbohydrates are not utilized: erythritol, L-arabinose, adonitol, β-methyl-xyloside, L-sorbose, dulcitol, sorbitol, α-methyl-D-mannoside, α-methyl-D-glucoside, melibiose, inulin, melezitose, D-raffinose, amidon, glycogen, D-fucose, D-arabitol, and L-arabitol. Nitrate is not reduced. Esculin, tyrosine, and casein are hydrolyzed but not urea or starch. The CAMP reaction is negative. DNase activity is present. Activities of pyrazinamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, acid phosphatase, phosphoamidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-glucosaminidase, α-mannosidase, and α-fucosidase are present whereas activities of pyrrolidonyl arylmidase, chymotrypsin, α-galactosidase, and β-glucuronidase are not observed.

The cell wall contains ornithine as diamino acid, the peptidoglycan type is B2α ([L-ornithine]D-glutamic acid-D-ornithine), and of the glycolyl type. C15:0*ai*, C16:0*i*, and C17:0*ai* are the predominant cellular fatty acids. Strains are resistant to vancomycin but susceptible to teicoplanin. Isolated from human clinical specimens. The type strain DMMZ 1710 has been deposited in the culture collection of the University of Göteborg, Sweden, as strain CCUG 38312.

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