Arabinose with LL-Diaminopimelic Acid in the Cell Wall of an Aerobic Coryneform Organism Isolated from Human Skin

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INTRODUCTION

The human cutaneous aerobic diphtheroids have often been classified in the genus *Corynebacterium* and various specific names have been proposed in the past. However, these organisms show such a bewildering variety of colonial, morphological and biochemical characteristics that they now warrant a detailed taxonomic study in order to determine their generic status.

Following the work of Cummins & Harris (1956), analysis for wall components has become a valuable taxonomic weapon. It is generally accepted that most pathogenic and commensal strains of corynebacteria of animal origin characteristically possess *meso*-diaminopimelic acid (*meso*-DAP) and arabinogalactan in their walls (Cummins, 1971): such walls are classed as type IV (Lechevalier & Lechevalier, 1970).

This paper describes six organisms with a coryneform morphology, which have LL-diaminopimelic acid (LL-DAP) and arabinose as their major wall components.

METHODS

Strains were received by courtesy of Professor R. van Furth, University Hospital, Leiden, The Netherlands, and were derived from a survey of the skin flora of dialysis and transplant patients (Noble, Rebel & Smith, 1974).

All strains were inoculated on 5% (v/v) horse serum agar plates and incubated aerobically for 48 h at 37 °C. Colonial characteristics were noted and the coryneform morphology confirmed by Preston and Morrell's modification of the Gram stain (Cowan & Steel, 1965). Batch culture was carried out at 37 °C with shaking in 1 l Erlenmeyer flasks containing brain-heart infusion broth (Oxoid). Cells were harvested in late-exponential growth.

The method of preparation of purified walls, hydrolysis conditions and chromatographic techniques for amino acids, amino sugars and neutral sugars, were those described by Cummins & Johnson (1971). The biochemical tests used were those of Cowan & Steel (1965) or Somerville (1973). Thin-layer chromatography for the detection of mycolic acids was carried out on dried whole cells using the acid methanolysis technique of Minnikin, Alshamaony & Goodfellow (1975*a*).

RESULTS

A total of 136 aerobic diphtheroids were subjected to wall analysis. The majority of strains possessed *meso*-DAP and arabinogalactan, but six strains were isolated in which only LL-DAP, arabinose and mannose were found as major components. These six isolates were obtained from different patients and from four different skin sites (toewebs, nose, shin and chest). In none of the patients was a strain isolated from more than one skin site. Plate

Table 1. *Wall composition of the six strains*

	Strain					
	D134	D426	D559	D635	D697	D716
LL-DAP	++	++	++	++	++	++
Alanine	+++	+++	+++	+++	+++	+++
Glutamic acid	++	++	++	++	+	+++
Glycine	++	++	++	++	+++	+++
Glucosamine	+++	+++	+++	+++	+++	+++
Galactosamine	—	++	—	+++	—	—
Muramic acid	++	++	++	++	++	++
Arabinose	+++	+++	+++	+++	+++	+++
Mannose	++	++	++	++	++	++
Galactose	—	—	—	—	—	—

The amount present is indicated as increasing from + to +++ ; —, not detected.

counts carried out by Noble *et al.* (1974) showed that four of the strains were present in sufficient numbers for them to be considered as resident members of the cutaneous flora.

Colonial morphology on 5% (v/v) horse serum agar was the same for all strains: a convex, shiny, white colony approximately 1 to 2 mm in diameter was obtained after 48 h at 37 °C. The microscopic appearance was of a small, non-motile, Gram-positive, pleomorphic bacillus showing snapping division and a strong tendency to clump. Metachromatic granules were not seen.

Strains were uniformly negative in the following tests: arginine hydrolysis; production of H₂S from cysteine; gelatin liquefaction; fluorescence on '199' medium; production of acid from glucose, galactose, fructose, maltose, sucrose, lactose and salicin. They were resistant to Furoxone (100 µg disc, Oxoid), growth was not enhanced by 0.1% Tween 80, and did not occur on MacConkey agar. All strains were catalase positive, oxidase positive, grew well under anaerobic conditions and on Hoyles tellurite medium, and in the presence of 7.5% (w/v) sodium chloride. The six strains varied in the biochemical characteristics of starch hydrolysis, nitrate reduction, urease activity and lipolysis of Tween 80.

Amino acids, amino sugars and neutral sugars detected in hydrolysates of isolated walls were identified by comparison with reference compounds (Table 1). No mycolic acids were detected by the acid methanolysis technique.

DISCUSSION

The morphological, cultural and biochemical features of these organisms are typical of those found for aerobic, cutaneous diphtheroids. However, the walls of the majority of diphtheroids contain *meso*-DAP, arabinose, galactose and usually mannose, with glycine either absent or present only in small amounts, whereas the six strains described possessed LL-DAP in conjunction with relatively large amounts of glycine. This observation is in agreement with published findings that in bacteria whose walls contain LL-DAP, peptidoglycan crosslinking occurs via glycine; but in bacteria whose walls contain *meso*-DAP there is usually no interpeptide bridge (Work, 1970; Schleifer & Kandler, 1972). Cummins & Johnson (1974) found LL-DAP in the walls of 41 out of 43 strains of *Propionibacterium acnes*, though in this case the accompanying sugars were glucose, mannose and galactose. These bacteria are found in large numbers on human skin, but are anaerobic in contrast to the six strains under discussion which grow very well aerobically. Other coryneforms that

contain LL-DAP are the *Arthrobacter simplex* group, but in these the major sugar is usually galactose (Keddie, Leask & Grainger, 1966). Arabinose in bacterial walls, accompanied by galactose and meso-DAP, is thought to be a characteristic of *Mycobacterium*, *Nocardia* and the animal corynebacteria. *Bergey's Manual of Determinative Bacteriology* (1974) suggests that this denotes a close relationship between the three genera.

The morphological, cultural and biochemical characteristics of the six strains closely resemble the description of *Corynebacterium pseudodiphtheriticum* in *Bergey's Manual of Determinative Bacteriology* (1974) but this species has a type IV wall and corynomycolic acids. The bacteria described here may be related to the animal corynebacteria since they possess an arabinose-mannose polymer, but perhaps for stereochemical reasons an arabinogalactan cannot co-exist with a peptidoglycan based on LL-DAP-glycine. Similar reasons might account for the absence of mycolic acids. No information is available as to whether mycolic acid-like lipids, occur in other LL-DAP containing organisms; and to date mycolic acids have only been associated with type IV walls (Cross & Goodfellow, 1973) though type IV walls have been found where mycolic acids were absent (Minnikin, Alshamaony & Goodfellow, 1975b).

It is suggested that though the six isolates cannot confidently be described as *Corynebacterium sensu stricto*, they are coryneforms of animal origin showing similarities to the corynebacteria of human skin and may belong to an, as yet, uncharacterized genus. Some qualitative and physiological differences occurred between the six strains, but an assessment of their taxonomic weight must await the isolation of more strains and more detailed characterization.

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