Characterization of *Corynebacterium* Group JK by Whole-cell Protein Patterns

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A total of 102 strains received as *Corynebacterium* 'group JK' were characterized by SDS-PAGE of their whole-cell proteins. Numerical taxonomy based on the protein pattern absorbance profiles indicated that 91 of the strains formed a cluster. Seventy strains isolated in the UK were identified as group JK, indicating the increasing detection of this group as opportunistic pathogens. Fine differences between strain patterns were visible but it was not possible to associate these with any particular clinical source.

INTRODUCTION

Corynebacterium spp. are common inhabitants of healthy human skin and mucous membranes. A number of highly antibiotic-resistant coryneform bacteria isolated from serious human infections, typically endocarditis, have been characterized and designated 'group JK' by the Special Bacteriology Section at the Centers for Disease Control (CDC), Atlanta, Ga., USA (Riley *et al.*, 1979). Group JK strains were characterized as catalase-positive, oxidase-negative, fermentative Gram-positive aerobic micro-organisms, which did not reduce nitrate. They were commonly resistant to ampicillin, cephalothin, chloramphenicol, erythromycin, gentamicin, penicillin G, streptomycin and tetracycline, and sensitive only to vancomycin.

Comparison of electrophoretic patterns of whole-cell proteins is useful in systematic studies of *Corynebacterium* (Jackman, 1982), and relationships between protein patterns have generally shown a high correlation with those from DNA–DNA hybridization (Owen & Jackman, 1982). The whole-cell protein patterns of 102 strains were compared. These were designated group JK by the CDC or isolated in the UK between 1983 and 1985 and described as similar to that group. The relationship of group JK to other *Corynebacterium* spp. is the subject of a separate study to be published elsewhere.

METHODS

Strains. *Corynebacterium* strains and their sources are listed in Table 1.

Preparation of protein extracts. Strains were cultured on a medium of tryptone soya broth (Oxoid), 30 g; yeast extract (Difco), 10 g; agar, 12 g and 5 ml Tween 80 per litre for 48 h at 37 °C. Cells (0.1 g) were harvested with a loop and lysed by incubation in 1 ml 0.1% (w/v) lysozyme for 3 h at 37 °C. The lysate was heated at 100 °C in the sample treatment buffer of Laemmli (1970) for 10 min and centrifuged for 10 min at 10000 *g*. The supernatant was stored at –20 °C before electrophoresis.

Electrophoresis. Samples were separated by SDS-PAGE in 10% (w/v) polyacrylamide gels (Laemmli, 1970) and standardized by the method of Jackman (1985).

Analysis of patterns. Patterns were scanned with a densitometer (Joyce–Loebl, Chromoscan 3) fitted with a 530 nm filter and a 3.0 × 0.1 mm slit aperture. Absorbance values were recorded at 0.2 mm intervals along the gel using a microcomputer (Sage IV, TDI Bristol, UK) interfaced with the densitometer. The patterns were corrected for gel-to-gel variation using a replicate bacterial sample (C454) run on each gel as a calibration pattern. Ten peaks were selected at approximately even intervals along the calibration pattern spanning a molecular mass range of 20

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Table 1. *Corynebacterium* sp. strains

Laboratory no.	Source	Laboratory no.	Source
C9, C10, C21, C27, C28, C29	NCTC A16/66, A17/66, A9/73, A113/74, A136/74, A137/74. Described in Athalye <i>et al.</i> (1984, 1985). C28 is NCTC 11914.	C429, C430, C433, C434, C435, C436, C437, C438, C439, C440, C441, C442, C476, C477, C478, C479, C483, C485, C486, C487, C488, C489, C490, C492, C508	Royal Marsden Hospital, London, UK. C428 is NCTC 11917. C483 is NCTC 11915 and K411 in Kerry-Williams & Noble (1984).
C63, C65, C67	L. Tomkins, University Hospital, Seattle, Wash., USA. Described in Athalye <i>et al.</i> (1984, 1985); Stamm <i>et al.</i> (1979).	C444	PHL, Leicester Royal Infirmary, UK
C105, C204, C205, C226, C428	Royal Marsden Hospital, London, UK	C445, C446, C447, C448, C449, C451, C452, C453, C454, C455, C456, C457, C458, C459, C461, C462, C463, C464	R. Weaver, CDC, Atlanta, Ga., USA. Described in Riley <i>et al.</i> (1979). C454 is NCTC 11913.
C137	University College Hospital, London, UK	C467, C468, C469, C470, C471	Walton Hospital, Liverpool, UK
C206, C207	St Paul's Hospital, London, UK	C472, C494, C495, C496, C497, C498, C499	Institute of Child Health, London, UK
C217	Herts. and Essex Hospital, Bishops Stortford, UK	C473	Department of Microbiology, University of Bristol, UK
C218, C225	Lister Hospital, Stevenage, UK	C475, C493, C501, C510	Southmead General Hospital, Bristol, UK
C220, C419, C420	Kings College Hospital, London, UK	C484	St Helier Hospital, Carshalton, UK
C221, C426, C506	Bradford Royal Infirmary, UK	C502	London College of Surgeons, UK
C222	RAF Institute of Pathology and Tropical Medicine, Aylesbury, UK	C504, C511, C512, C513	The London Hospital, UK
C227	North Devon District Hospital, Barnstable, UK	C545	Isle of Thanet District Hospital, Margate, UK
C416, C417, C421, C422	Mayday Hospital, Croydon, UK	C507	Withington Hospital, Manchester, UK
C424	Northwick Park Hospital, London, UK		

to 100 kDa. The distances between each of the calibration peaks were measured on every gel and a single pattern was chosen to act as reference for the whole study. Then the corresponding sections of the bacterial patterns on each gel were corrected by expansion or contraction to the reference distances. The length was corrected by three point quadratic interpolation (Jackman *et al.*, 1983).

The absorbance traces of the patterns were compared with the correlation coefficient and UPGMA clustering, as was found useful in previous studies (Jackman *et al.*, 1983; Kersters & De Ley, 1975). A computer program in Pascal to do these procedures is available from the corresponding author.

RESULTS AND DISCUSSION

Representative whole-cell protein patterns are shown in Fig. 1. The numerical comparison of the patterns is shown as a dendrogram in Fig. 2. All but nine of the 102 strains formed a single cluster linked at >0.70 correlation. The strains clustered at this level appeared to match most closely with a visual assessment of the electropherograms. Further taxonomic measures are required to confirm the classification obtained from protein patterns. The mean correlation between replicate samples run on each of the 12 gels used was 0.93 after correction for gel-to-gel variation as described in Methods. Since many of the strains had been identified only by their coryneform morphology and antibiotic resistance, the high level of homogeneity observed was remarkable, particularly in view of the diversity amongst both reference *Corynebacterium* spp. and isolates from human skin found in a previous study (Jackman, 1982). The level of similarity

Fig. 1. Whole-cell protein patterns of *Corynebacterium* group JK. Molecular weight markers (MWM) were trypsin inhibitor (20100), carbonic anhydrase (30000), ovalbumin (43000), albumin (67000), phosphorylase b (94000). (a) Gel 651. Lanes: 1, C222; 2, C206; 3, C220; 4, C221; 5, C227; 6, C454; 7, MWM; 8, C416; 9, C426; 10, C205; 11, C433. (b) Gel 652. Lanes: 1, C469; 2, C476; 3, C477; 4, C479; 5, C490; 6, C454; 7, MWM; 8, C492; 9, C495; 10, C496; 11, C497; 12, C463.

Fig. 2. Dendrogram of *Corynebacterium* group JK based on comparison of whole-cell protein patterns using the correlation coefficient and UPGMA clustering.

among the group JK strains was similar to that between reference strains of *C. diphtheriae* (Jackman, 1982), which confirms the coherence of the group, as found by Riley *et al.* (1979). All group JK patterns were characterized by a prominent band of about 45 kDa; however, there was much fine strain variation particularly in the high molecular mass range of the gel. Some of these pattern variants formed subclusters in the dendrogram, e.g. a cluster of 11 strains (C417 to C498) linking at >0.75 ; however, other observable variants did not cluster. This may be due to an inherent weighting of the correlation coefficient towards the bigger features of the patterns. As far as could be ascertained, the observable strain variants showed no particular association with clinical source.

This study provides evidence that there are a considerable number of opportunistic infections recently detected in the UK which are associated with strains of the species described as *Corynebacterium* group JK by the CDC. The commonest sources of group JK isolates were blood (45%), infected lines or shunts (9%), urethra or urine (7%), ulcers and abscesses (4%) and heart (3%). The commonest associated conditions were leukaemia (16%) and renal dialysis (9%). Twelve isolates were from the skin or vagina of cancer patients. The clinical picture of group JK as an opportunistic pathogen of immuno-compromised patients was confirmed in the UK, but with perhaps fewer cases of endocarditis than expected.

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