Staphylococcal whole-cell polypeptide analysis: evaluation as a taxonomic and typing tool

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Summary. Whole-cell-polypeptide profiles obtained by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) were used in conjunction with the API-Staph® technique to identify different strains of *Staphylococcus aureus*, *S. epidermidis*, *S. saprophyticus* and *S. capitis*. Complete concordance of results from both techniques was achieved with all strains examined. Visual analysis of the polypeptide patterns and comparison by use of the coefficient of Dice showed minor differences in band pattern between strains of the same species but each species produced a pattern distinguishable from that of any other. The results suggest that although SDS-PAGE can be used to identify staphylococcal species, this type of analysis will not readily provide the basis for a typing method.

Introduction

Polyacrylamide gel electrophoresis (PAGE) of bacterial polypeptides solubilised by treatment with sodium dodecyl sulphate (SDS) has been used successfully as a taxonomic tool by several workers. Kersters and De Ley (1975) have shown that densitometric analysis of stained tube gels provides data suitable for analysis by the techniques of numerical taxonomy. The introduction of slab gels (Studier, 1973) has facilitated the comparison of samples and this technique, combined with the buffer systems introduced by Laemmli (1970), has been widely used in the taxonomic analysis of bacteria at the level of species and genera (Jackman, 1982; Kersters, 1985) and in the typing of strains within a species (Barenkamp *et al.*, 1981).

Kloos and Schleifer have proposed a taxonomic scheme for staphylococci (Kloos and Schleifer, 1975; Schleifer and Kloos, 1975) and DNA-hybridisation data, reviewed by Kloos (1980), and the results of numerical taxonomy studies (Feltham, 1979) agree well with their proposals. This paper describes the results of PAGE of polypeptides of four staphylococcal species. The aim of the study was to evaluate the usefulness of the technique as a taxonomic tool in this genus, and to establish whether typing schemes could be established based upon data from the polypeptide analysis of coagulase-negative species.

Materials and methods

Preparation of samples

Staphylococcal strains were isolated from the anterior nares and scalp of healthy volunteers. Strains were identified to genus level by the methods described by Cowan and Steel (Cowan, 1974), by coagulase testing, and by the API Staph® technique (API Laboratory Products, Basingstoke, Hants (Brun *et al.*, 1978). Species identified as *S. aureus*, *S. capitis*, *S. epidermidis* or *S. saprophyticus* were stored at +4°C. Other strains were obtained from Dr W. Kloos, Raleigh, NC, USA—they included *S. capitis* strains ATCC 27840 and 27841 and *S. saprophyticus* strains CCM 883 and KL 122—and from Dr R. R. Marples, Central Public Health Laboratory, Colindale, London, who supplied *S. capitis* strain 199672, *S. saprophyticus* strain 100798 and *S. epidermidis* strain 100620 (described by Marples and Richardson, 1981). Cultures for electrophoresis were prepared by inoculating strains into 250-ml volumes of nutrient broth which were incubated with shaking at 37°C in an orbital incubator overnight. The organisms were then sedimented by centrifugation at 3000 *g* for 15 min. The supernate was discarded and the pellet was suspended and microcentrifuged (Microfuge; Beckman, USA) for 5 min. After removal of the supernate, a volume of sterile distilled water equivalent to the pellet volume was added. The samples were then placed in a water bath containing melting ice and were subjected to sonication for 5 min from an ultrasonic probe (Rapidis 150, Ultrasonics Ltd, Shipley, England) emitting 45W at maximum power, before being stored at –70°C.

SDS-PAGE

Slab gels, 160 mm × 168 mm × 1.5 mm, of polyacrylamide 10% w/v (Serva, Cambridge) with a 10-mm poly-

acrylamide 3.6% w/v stacking gel, were run in a BRL gel apparatus, model V16-2 (BRL, Gaithersburg, MD, USA). Buffers used were as described by Laemmli (1970). Samples were mixed in 2:1 proportions with 0.5M Tris-HCl, pH 6.8, containing SDS 6% w/v, glycerol 30% w/v, 2-mercaptoethanol 15% w/v and bromophenol blue 0.001% w/v, and boiled for 5 min before application to the gels; a 10- μ l volume containing 70–75 μ g of protein estimated by quantitative densitometry was applied to each well. The gel was run at a constant current of 30 mA until the bromophenol blue was seen leaving the bottom of the gel. This normally took 3–4 h. The gel was then removed and stained in Coomassie Brilliant Blue R (Sigma Chemical Co. Ltd, Poole) 0.25% w/v in methanol:acetic acid:water (45:10:45 by volume) and destained in the same solvent mixture before being swollen to its original size in acetic acid 7% v/v. Mol.-wt marker polypeptides (Pharmacia, Uppsala, Sweden) were run in the same gels to allow the estimation of mol. wts.

Densitometry was done with an LKB Ultrosan Laser Densitometer with Gelscan software.

Comparison of polypeptide profiles

The average similarity between two strains was assessed by the coefficient of Dice (1945), whereby

$$\text{average percentage similarity (\%S)} = \frac{\text{number of matching bands} \times 2}{\text{total number of bands in both strains}} \times 100$$

The calculation of similarity was based upon visual comparisons made independently by two individuals and preparations from all strains were run several times to confirm the results.

Results

Disruption of staphylococci by sonication and treatment with SDS and 2-mercaptoethanol was assessed by microscopy of gram-stained films and by electronmicroscopy after negative staining. About 80% of cells in a preparation were disrupted by the method used and the complexities of the polypeptide profiles obtained after Coomassie blue staining were similar to those obtained after cell disruption by lysostaphin treatment (Saleh and Freer, 1984). Because electropherograms of uninoculated nutrient broth failed to give bands after Coomassie blue staining, all bands in gels were considered to be of staphylococcal origin.

A total of 56 strains of *S. aureus*, 47 strains of *S. epidermidis*, 18 strains of *S. capitis* and 9 strains of *S. saprophyticus* was studied. Gels showed 30–50 bands after staining with Coomassie blue and typical results are shown in the figures. Each of the four staphylococcal species gave a characteristic and distinct band profile (fig. 1) and there was complete concordance between the type of gel

Fig. 1. Whole-cell-polypeptide profiles of two different strains of each staphylococcal species: strains A and B, *S. aureus*; strains C and D, *S. capitis*; strains E and F, *S. epidermidis*; strains G and H, *S. saprophyticus*.

Table. Average percentage similarity (%S) of whole-cell-polypeptide profiles of staphylococcal strains shown in Fig. 1

Strains compared		%S
<i>S. aureus</i>	<i>S. aureus</i>	92.75
<i>S. aureus</i>	<i>S. capitis</i>	22.45
<i>S. aureus</i>	<i>S. epidermidis</i>	31.10
<i>S. aureus</i>	<i>S. saprophyticus</i>	28.35
<i>S. capitis</i>	<i>S. capitis</i>	90.80
<i>S. capitis</i>	<i>S. epidermidis</i>	36.95
<i>S. capitis</i>	<i>S. saprophyticus</i>	39.45
<i>S. epidermidis</i>	<i>S. epidermidis</i>	97.25
<i>S. epidermidis</i>	<i>S. saprophyticus</i>	37.80
<i>S. saprophyticus</i>	<i>S. saprophyticus</i>	94.75

profile and the species identification made by API Staph and by other methods. The profiles of strains obtained in Aberdeen and of those obtained from Marples and Kloos were also concordant. Estimations of the Dice coefficients of similarity gave quantitative assessments of the visual judgement in terms of the presence of co-migrating bands, and confirmed the distinctive nature of the band patterns (table). Calculation of the Dice coefficients from band position data obtained by densitometry gave similar results. Little variation of band pattern

between strains of the same species was noted; > 90% of the bands detected were present in similar amounts in all strains of each species investigated. Thus, in the gel shown in fig. 2, where nine strains of *S. aureus* were compared, 41 bands were common to all nine strains and only four bands were not present in all strains. Strains B and D lacked a band of mol.wt 70×10^3 , strain I lacked a band of mol.wt 49×10^3 but had a new band migrating slightly faster; strain C had a band of mol.wt 36×10^3 and strain I one of 25×10^3 , neither of which was present in the remaining strains. Similar results were obtained with 18 strains of *S. capitis*, 47 strains of *S. epidermidis* and 9 strains of *S. saprophyticus* (data not shown).

Discussion

The complete concordance of whole-cell SDS-PAGE band pattern and identification of staphylococci according to the scheme of Kloos and Schleifer (1975) provides further support for their taxonomic proposals, which already agree well with DNA hybridisation data (Kloos, 1980).

Only minor variations in band pattern were observed between strains of any of the four species analysed, and it seems clear that SDS-PAGE of polypeptides in whole-cell extracts will not readily

Fig. 2. Whole-cell-polypeptide profiles of nine different strains of *S. aureus* (lanes A-I). Mol. wts (10^3) are indicated.

provide data suitable for the establishment of typing schemes. These findings confirm and extend those of Krikler *et al.* (1986) who showed that, in the case of *S. aureus*, little band variation in SDS-PAGE profiles occurred from strain to strain. However, these workers showed that the analysis of proteins exported from *S. aureus* could be used to

form the basis of a typing scheme, and we have initiated studies to evaluate this approach with other staphylococcal species.

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