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Availability of Use of Total Extracellular Proteins in SDS-PAGE for Typing *Staphylococcus aureus* and Coagulase-Negative Staphylococci

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Abstract: A total of nineteen Staphylococcus references, nine of which are *Staphylococcus aureus* and ten coagulase-negative Staphylococci (CNS), were characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Whole-cell protein (WCP) profiles obtained by SDS-PAGE were compared with banding patterns produced by SDS-PAGE of total extracellular proteins (TEP). No report has yet been issued which explains Staphylococcus typing by SDS-PAGE of extracellular proteins. SDS-PAGE of whole-cell extracts did not usefully distinguish different isolates of Staphylococci. Our preliminary results suggest that SDS-PAGE of total extracellular proteins provides additional criteria and characteristic banding patterns for the study of the epidemiology and characterization of Staphylococci. SDS-PAGE of extracellular proteins provided a rapid, reproducible and discriminative method for characterization of Staphylococci which is easy to perform and interpret.

Key Words: *Staphylococcus aureus*, coagulase-negative staphylococci (CNS), protein profiles, typing.

SDS-PAGE'de Hücre Dışı Proteinlerinin *Staphylococcus aureus* ve Koagülaz Negatif Stafilokokların Tiplendirimi İçin Kullanım Uygunluğu

Özet: Dokuz tanesi *Staphylococcus aureus* ve 10 tanesi koagülaz negatif stafilokok (CNS) olmak üzere toplam 19 adet referans Stafilokoklar sodyum dodesil sülfat-poliakrilamid jel elektroforezde (SDS-PAGE) karakterize edilmiştir. SDS-PAGE'de toplam hücre proteinleri (WCP) kullanıldığı zaman elde edilen protein bant patterni, SDS-PAGE'de toplam hücre dışı proteinler kullanılarak elde edilen protein bant patternleri ile karşılaştırılmıştır. Şu ana kadar SDS-PAGE'de hücre dışı proteinler kullanılarak Stafilokokların tiplendirimine ait herhangi bir çalışmaya rastlanmamıştır. Çalışmalarımızın sonucunda SDS-PAGE'de toplam hücre dışı proteinlerin kullanılmasının Stafilokokların karakterizasyonu ve epidemiyolojilerinin çalışılmasında karakteristik bant paternleri oluşturduğu için ek bir avantaj sağladığı gözlemlenmiştir.

Anahtar Sözcükler: *Staphylococcus aureus*, koagülaz negatif staphylococci (CNS), protein profilleri, tiplendirme.

Introduction

Infection with coagulase negative Staphylococci (CNS) and *Staphylococcus aureus* is an increasing problem in hospital practice, but no effective single typing method exists with which to investigate the epidemiology of these infections. To date many different methods have been used for typing Staphylococci, including serotyping (1), capsular typing (2), bacteriophage typing (3), antibiogram and biotyping (4, 5), analysis of cellular fatty acids (6) and molecular genotyping methods or DNA-based techniques (7-10). Analysis of whole cell profiles by SDS-PAGE has recently been established as a useful method for the identification of Staphylococcal and Bacillus species (11-13) and the investigation of individual strains of coagulase negative Staphylococci (14). However, low selection at the strain level and high selection at the species level have been observed (11, 15, 16). Furthermore, it is known that SDS-PAGE of whole cell extracts cannot provide suitable data at the strain level (11, 16).

To date, SDS-PAGE of whole-cell proteins has been used for typing Staphylococci, but no report has been issued as yet regarding typing by SDS-PAGE of extracellular proteins. In our research, SDS-PAGE of extracellular proteins was used successfully to type isolates of Staphylococci, and the results of extracellular proteins were compared with those of whole-cell proteins.

Materials and Methods

Bacterial strains and media. All cultures were grown at 35°C for 24 h in BHI (brain heart infusion, Difco) agar and propagated at least twice before use. The reference bacteria used in our project were kindly provided by the following researchers: *S. aureus* ATCC 29740, *S. sciuri* ATCC 29062, *S. simulans* ATCC 27848, *S. haemolyticus* ATCC 29970, *S. xylosus* ATCC 29971, *S. chromogenes* ATCC 43764 and *S. warneri* ATCC 27836, by Dr. Jeffry L. Watts (Luisiana State University Agricultural Center, Homer, LA, USA); *S. aureus* NCTC 8356, *S. aureus* NCTC 8357, *S. aureus* NCTC 8511, *S. aureus* NCTC 10033 and *S. epidermidis* ATCC 12228, by Numan Erdal (Refik Saydam Hıfzısıhha Institute, Ankara, Turkey); *S. aureus* ATCC 43300 and *S. aureus* NCTC 8325, by Dr. Yutaka Tajima (Saga Medical School, Hospital Nasephima, Saga, Japan); *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 14990, by Dr. Elisabeth Davidson (Arizona State University, Department of Zoology, Tempe, Arizona, USA); *S. aureus* NCTC 8530 (Cowan 1), by Dr. Haluk Ataoğlu (Ankara University, School of Medicine, Microbiology and Clinical Microbiology Department, Ankara, Turkey); *S. saprophyticus* ATCC 15305, by Dr. Ahmet Basustaoğlu (Gülhane Military Medical Academy, Department of Infectious Diseases and Clinical Microbiology, Ankara, Turkey); *S. simulans* bv. *staphylolyticus*, from Dr. Junji Sakurada (Jikei University, School of Medicine, Department of Bacteriology, Tokyo, Japan).

Preparation of whole cell proteins. For each culture, a loopful of overnight growth from a BHI agar plate was suspended in 15 ml BHI broth and incubated in a rotated

incubator for 7 h (at 35°C, 150 rpm). Samples were then transferred into eppendorf tubes and centrifuged for 3 min at 12100 rpm, and the collected cells were washed three times with distilled water. The washed cells were stirred after the addition of 25µl SDS sample buffer (0.06 M Tris, 2.5% Glycerol, 0.5% SDS, 1.25% ß-mercaptoethanol) and the proteins were denatured in boiling water for 5 min. Supernatant was then centrifuged again for 3 min at 12100 rpm, collected in an eppendorf tube and kept at -50ºC until electrophoresis was carried out.

Preparation of extracellular proteins. The method of Wessel and Flugge (17) was used with a few modifications. The culture supernatants were passed through a cellulose acetate membrane filter (Sartorius) with a diameter of 0.2 µm and stored at -50°C until electrophoresis was carried out. Methanol-chloroform precipitation was performed. 400µl methanol, 200µl chloroform and 300µl distilled water were added over 500µl of sample, and the mixture were centrifuged for 3 min at 10700 rpm. After centrifugation, before it went into intermediate phase, the supernatant was removed carefully and after the addition of 300µl methanol and stirring, it was centrifuged again at 10700 rpm. After the supernatant was removed, the precipitated proteins were dried by air and stirred after the addition of 25µl SDS-sample buffer. Afterwards, proteins were denatured by being kept in boiling water for 5 min.

SDS-PAGE. Solubilized proteins were analysed by SDS-PAGE. This method involved a 2 cm layer of 4% acrylamide stacking gel and a 10 cm layer of 10% acrylamide separating gel. A Sigma wide range marker was used as molecular weight standard in SDS-PAGE. Electrophoresis was performed with a discontinuous buffer system in a BRL gel apparatus model V16-2BRL, Gaithersburg MD, USA. The gel was run at a constant current of 35mA until the bromophenol blue marker had reached the bottom. Gels were then stained with Coomassie Brilliant Blue (18).

Results

Analysis of different strains of *S. aureus* and different species of CNS by SDS-PAGE gave reproducible whole-cell polypeptide profiles (Figure 1). However, only minor variations in band patterns were observed between the strains of *S. aureus* and species of CNS analyzed, and it seems clear that SDS-PAGE of polypeptides in whole-cell extracts will not readily provide data suitable for the establishment of the typing scheme. In contrast, SDS-PAGE of extracellular proteins gave characteristic profiles which were used to discriminate different strains of *S. aureus* and species of CNS.

As far as the band profiles of TEPs in SDS-PAGE were concerned, a common characteristic bands of 6.7 kDa were seen in *S. aureus* strains but not in CNS species. Furthermore, 21.3 and 64 kDa characteristic bands in strain 1; a high level expression of 62 kDa band in strain 2; 9.6, 15.2 and 20.0 kDa dominant bands in strain 3; a band over 205 kDa and 22.8 kDa protein bands in strain 7; twin bands of 21.7 and 22.5 kDa in strain 4 and of 22.6 and 23 kDa in strain 6; and a low level expression

of 14.5 kDa protein as well as the presence of 40.2 kDa twin bands in strain 9 provided a better distinction than SDS-PAGE of whole-cell protein profiles and, in this way, high level differences among *S. aureus* strains and CNS species which may lead to differentiation were observed (Figure 2). No characteristic bands were observed in strains 5 and 8 even when SDS-PAGE of extracellular proteins was used.

Discussion

In this study, we evaluated the suitability of total extracellular proteins (TEPs) and whole cell proteins (WCPs) in SDS-PAGE as a routine typing system for Staphylococci at both the species and the strain levels, and we compared the utility of TEPs with that of WCPs. Our results and those of previous studies (11, 19, 20) clearly show that electrophoretic methods can provide valuable epidemiological information that may be used in isolation. It was also concluded that SDS-PAGE of polypeptides of whole-cell extracts did not readily provide data for the establishment of typing schemes (11, 15, 16). We also agree with Clink and Pennington (11) that the patterns given by whole-cell polypeptides of different strains of *S. aureus* and different species of CNS are very similar and some differences were very minor. Our findings confirm and extend those of Krikler (19), who showed that, in the case of *S. aureus*, little band variation in

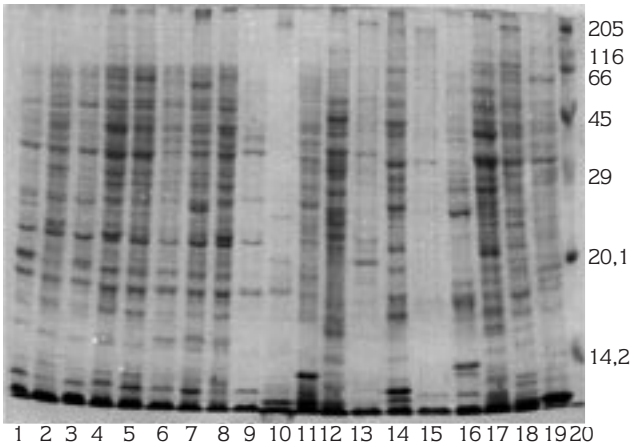

Fig. 1. SDS-PAGE of whole-cell proteins of *S. aureus* strains and CNS species. Line 1; *S. aureus* NCTC 8530 (Cowan 1), 2; NCTC 8325, 3; NCTC 8356, 4; NCTC 8357, 5; NCTC 8511, 6; NCTC 10033, 7; ATCC 6538, 8; ATCC 29740, 9; ATCC 43300, 10; S. epidermidis ATCC 14990, 11; ATCC 12228, 12; *S. sciuri* ATCC 29062, 13; *S. saphrophyticus* ATCC 15305, 14; *S. haemolyticus* ATCC 29970, 15; *S. simulans* ATCC 27848, 16; *S. simulans biovar staphylolyticus* NRRL B2628, 17; *S. chromogenes* ATCC 43764, 18; *S. xylosus* ATCC 29971, 19; *S. warneri* ATCC 27836, 20; molecular weight marker of SDS-PAGE in kDa (Sigma wide-range marker).

SDS-PAGE profiles occurred from strain to strain. However, these workers showed that the analysis of extracellular proteins from *S. aureus* and CNS could be used to form the basis of a typing scheme, and therefore the work was carried out using culture supernatants for analysis.

The development of new methods for typing Staphylococci is desirable since existing methods often give ambiguous or misleading results. In contrast to SDS-PAGE of polypeptides of whole-cell extracts, total extracellular proteins of Staphylococci in SDS-PAGE gave characteristic banding patterns that could be used to differentiate *S. aureus* at the strain level and CNS at the species level, and this method has the potential to be developed into an alternative or ancillary typing method for routine use. Our new typing system is able to discriminate between distinct strains of *S. aureus* and species of CNS according to the formation of characteristic protein bands specific to each species and strain.

We can conclude that the bands obtained in SDS-PAGE of whole-cell proteins were difficult to interpret. The protein bands in the SDS-PAGE of whole-cell proteins gel was also complex and less discriminatory than SDS-PAGE of extracellular proteins. On the other hand, the patterns obtained by TEP profiles were much easier to interpret because of the presence of characteristic protein bands, and they may provide a rapid procedure for characterization of Staphylococci because only one hour is required for the extraction of 7 h rotated culture, a great time advantage as compared to other typing tools.

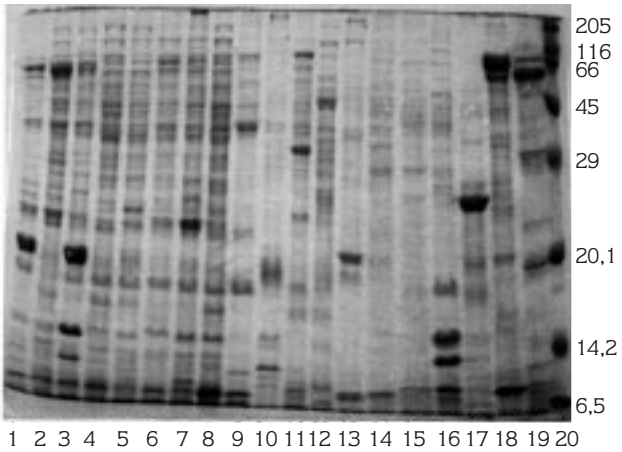

Fig. 2. SDS-PAGE of total extracellular proteins of *S. aureus* strains and CNS species. Line 1: *S. aureus* NCTC 8530 (Cowan 1), 2: NCTC 8325, 3: NCTC 8356, 4: NCTC 8357, 5: NCTC 8511, 6: NCTC 10033, 7: ATCC 6538, 8: ATCC 29740, 9: ATCC 43300, 10: *S. epidermidis* ATCC 14990, 11: ATCC 12228, 12: *S. sciuri* ATCC 29062, 13: *S. saphrophyticus* ATCC 15305, 14: *S. haemolyticus* ATCC 29970, 15: *S. simulans* ATCC 27848, 16: *S. simulans biovar staphylolyticus* NRRL B2628, 17: *S. chromogenes* ATCC 43764, 18: *S. xylosus* ATCC 29971, 19: *S. warneri* ATCC 27836, 20: molecular weight marker of SDS-PAGE in kDa (Sigma wide-range marker).

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