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EXPERIMENTAL ARTICLES

Characterization of *Staphylococcus* species by SDS–PAGE of Whole-Cell and Extracellular Proteins[1]

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Abstract—In this study, a total of fifteen staphylococcal strains belonging to different species were characterized by whole-cell and extracellular protein profiles using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The results are presented as dendrograms after quantitative analysis of the band patterns with a computer program. Visual inspection of protein bands and cluster analysis of protein patterns of to be used 15 strains, representing 10 *Staphylococcus* species, showed that whole-cell and extracellular protein profiles differed in several protein bands in *Staphylococcus aureus*, *S. epidermidis*, *S. simulans*, and other species of *Staphylococcus*; however, the differences were insufficient for reliable differentiation of *Staphylococcus* species by the SDS–PAGE method.

Key words: characterization, staphylococci, SDS–PAGE, protein.

In recent years, strains of *Staphylococcus aureus* and other strains of staphylococci have caused increasingly severe problems of nosocomial infection in many countries around the world [1]. Nevertheless, no effective universal typing method exists to conduct research on the epidemiology of these infections. Many different methods have been used for typing staphylococci. Serotyping [2], capsular typing [3], bacteriophage typing [4], pulsed-field gel electrophoresis [5–7], antibiogram analysis and biotyping [8, 9], and analysis of cellular fatty acids [10] are some of the researches conducted on these bacteria. Protein electrophoresis has been of great value in bacterial classification and identification [11–13]. A second level of information about a cell, after the complete nucleotide sequence of the bacterial genome, is provided by cellular proteins, and different types of electrophoresis have been used to explore relationships at this level. Analysis of whole-cell protein profiles by SDS–PAGE has recently been established as a useful method for the identification of a number of bacteria [14–16], particularly of individual strains of coagulase-negative staphylococci [17, 18]. However, low specificity was observed at the strain level, despite the high specificity at the species level [19, 20]. It has been reported that SDS–PAGE of whole-cell extracts cannot provide reliable differentiation at the strain level [11, 19, 21].

According to the available data concerning this subject, SDS–PAGE of whole-cell proteins has been used for typing of staphylococci, but there are no reports regarding typing of staphylococci by SDS–PAGE of extracellular proteins. In our research, extracellular protein profiles obtained by SDS–PAGE of culture supernatant extracts were used for distinguishing *S. aureus* strains and other *Staphylococcus* species from each other, and the results were compared with those yielded by analysis of whole-cell protein patterns.

MATERIALS AND METHODS

Bacteria and growth conditions. All cultures were grown at 37°C for 24 h on BHI (Difco) agar (at least two culture transfers before use in the experiments). The test bacteria used in our study were provided by various researchers: *S. aureus* ATCC 29740, *S. sciuri* ATCC 29062, *S. simulans* ATCC 27848, *S. chromogenes* ATCC 43764, and *S. warneri* ATCC 27836 were from Dr. J.L. Watts (Louisiana State Univ. Agricultural Center, Homer, LA, USA); *S. aureus* NCTC 8511, *S. aureus* NCTC 10033, *S. epidermidis* ATCC 12228, and *S. haemolyticus* ATCC 29970 were from Dr. N. Erdal (Refik Saydarn Hifsisihha Institute, Ankara, Turkey); *S. aureus* ATCC 43300 was from Dr. Y. Tajima (Saga Medical School, Hospital Nasephima, Saga, Japan); *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 14990 were from Dr. E. Davidson (Arizona State University, Department of Zoology, Tempe, AZ, USA); *S. saprophyticus* ATCC 15308 and *S. xylosus* ATCC 29971 were from Dr. A. Basustaoglu (Gulhane Military Medical Academy, Infectious Diseases and Clinical Microbiology Department, Ankara, Turkey); and *S. simulans* biovar. *staphylolyticus* NRRL B2628 was from Dr. J. Sakurada (Jirkei Univ. School of Medicine, Department of Bacteriology, Tokyo, Japan).

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Preparation of whole-cell proteins. For each culture, a loopful of overnight growth from a BHI agar plate was suspended in 15 ml of BHI broth and incubated in a rotated incubator (150 rpm) for 7 h at 37°C. Samples were then transferred to 1.3-ml Eppendorf tubes and centrifuged for 3 min at 10000 *g*; the collected cells were washed three times with distilled water. After adding 25 μl of denaturing buffer containing 0.06 M Tris-HCl, 2.5% glycerol, 0.5% SDS, and 1.25% β-mercaptoethanol (pH 6.8), the cells were stirred, and the proteins were denatured in boiling water for 5 min [22]. After centrifugation for 3 min at 10000 *g*, the supernatant was put in an Eppendorf tube and kept at –50°C until electrophoresis was carried out.

Preparation of extracellular proteins. The method of Wessel and Flügge [23] was used with a few modifications. Culture supernatants were passed through cellulose acetate membrane filters (Sartorius) with a pore diameter of 0.25 μm and stored at –50°C until further use. Before electrophoresis, methanol–chloroform precipitation was performed by centrifugation of a mixture of 400 μl of methanol, 200 μl of chloroform, 300 μl of distilled water, and a certain amount of the culture liquid in Eppendorf tubes for 3 min at 12000 *g*. After centrifugation, the upper phase was removed, 300 μl of methanol was added, and the mixture was stirred and centrifuged again at 10700 rpm. Then the supernatant was removed and the precipitated proteins were dried at room temperature. Dried proteins were resuspended in 25 μl of the above-specified denaturing buffer in Eppendorf tubes, which were kept in boiling water for 5 min.

SDS–PAGE. Solubilized proteins were subjected to SDS–PAGE as described by Laemmli [22] in gel slabs 0.75 mm thick and 15 cm long (1.5 cm of 4% stacking gel and 13.5 cm of 10% resolving gel). Electrophoresis was performed with a discontinuous buffer system in a V16-2BRL apparatus (BRL, Gaithersburg, MD, USA). The gel was run at 35 mA until the bromophenol blue marker had reached the bottom of the gel. Gels were then stained with Coomassie Brilliant Blue R-250.

Data analysis. Gels were examined by the naked eye and the protein profiles were recorded as binary data, that is, 1 or 0. The resultant data were typed into the MINITAB program for input and analysis of binary data on an IBM computer. The similarity and relationship between the protein profiles of test strains were expressed in terms of a cluster dendrogram based on the unweighted pair group method with an arithmetic averages algorithm (UPGMA).

RESULTS AND DISCUSSION

Staphylococcal whole-cell protein profiles and total extracellular protein profiles obtained by SDS–PAGE are shown in Figs. 1 and 2. The protein profiles were inspected visually and compared with each other.

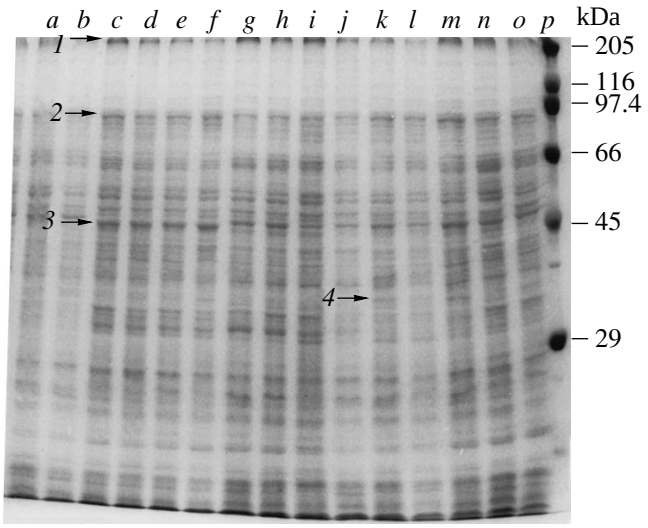

Fig. 1. SDS–PAGE of whole-cell proteins of *S. aureus* and other *Staphylococcus* species. Lanes: (*a*) *S. aureus* NCTC 8511; (*b*) NCTC 10033; (*c*) ATCC 6538; (*d*) ATCC 29740; (*e*) ATCC 43300; (*f*) *S. epidermidis* ATCC 14990; (*g*) ATCC 12228; (*h*) *S. sciuri* ATCC 29062; (*i*) *S. saprophyticus* ATCC 15308; (*j*) *S. haemolyticus* ATCC 29970; (*k*) *S. simulans* ATCC 27848; (*l*) *S. simulans* biovar. *staphylolyticus* NRRL B2628; (*m*) *S. chromogenes* ATCC 43764; (*n*) *S. xylosus* ATCC 29971; (*o*) *S. warneri* ATCC 27836; (*p*) molecular weight standards (kDa): 205 (myosin), 116 (β-galactosidase), 97.4 (phosphorylase B), 66 (bovine serum albumin), 45 (egg albumin), 29 (carbonic anhydrase).

SDS–PAGE of various strains of *S. aureus* and other *Staphylococcus* species produced reproducible whole-cell protein profiles (Fig. 1). The band (marked by *1*) at the top of each lane is the cell-surface protein of *S. aureus* strains and other strains of *Staphylococcus* spp. The profiles of all *S. aureus* strains and strains of other *Staphylococcus* species exhibited similar bands; the similarity was particularly manifested in the presence of bands *2*, *3*, and *7*. However, distinctions were observed between protein profiles of *S. aureus* strains and other strains of staphylococci. For instance, the protein profiles of strains *S. simulans* ATCC 27848, *S. simulans* biovar. *staphylolyticus* NRRL B2628, and *S. chromogenes* ATCC 43764 were similar to those of *S. aureus* strains and other staphylococci, but were distinguished by the presence of a single specific band *4*.

The extracellular protein patterns of *S. aureus* strains and other *Staphylococcus* species (Fig. 2) were also similar: *S. aureus* strains and other strains of staphylococci exhibited the presence of bands *5* and *10*. Strains *S. simulans* biovar. *staphylolyticus* NRRL B2628, *S. chromogenes*, *S. xylosus*, and *S. warneri* were similar to other staphylococcal strains, but were distinguished by the presence of intense bands *6* and *9*. Band *8* was observed in the profiles of *S. aureus* (lanes *c*, *d*, and *e*), *S. epidermidis* (lanes *f* and *g*), *S. sciuri* (lane *h*), *S. saprophyticus* (lane *i*), and *S. haemolyticus* (lane *j*).

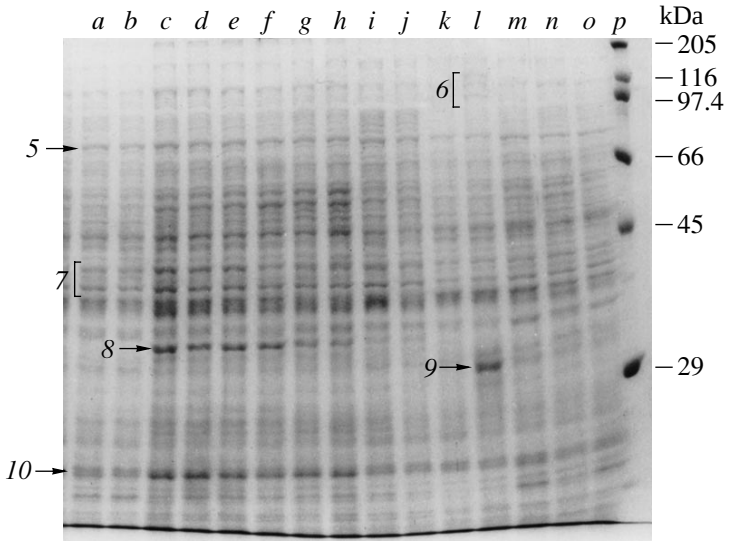

Fig. 2. SDS–PAGE of extracellular proteins of *Staphylococcus aureus* and other *Staphylococcus* species. Lanes: (*a*) *S. aureus* NCTC 8511; (*b*) NCTC 10033; (*c*) ATCC 6538; (*d*) ATCC 29740; (*e*) ATCC 43300; (*f*) *S. epidermidis* ATCC 14990; (*g*) ATCC 12228; (*h*) *S. sciuri* ATCC 29062; (*i*) *S. saprophyticus* ATCC 15308; (*j*) *S. haemolyticus* ATCC 29970; (*k*) *S. simulans* ATCC 27848; (*l*) *S. simulans* bv. *staphylolyticus* NRRL B2628; (*m*) *S. chromogenes* ATCC 43764; (*n*) *S. xylosus* ATCC 29971; (*o*) *S. warneri* ATCC 27836; (*p*) molecular weight standards (kDa): 205 (myosin), 116 (β-galactosidase), 97.4 (phosphorylase B), 66 (bovine serum albumin), 45 (egg albumin), 29 (carbonic anhydrase).

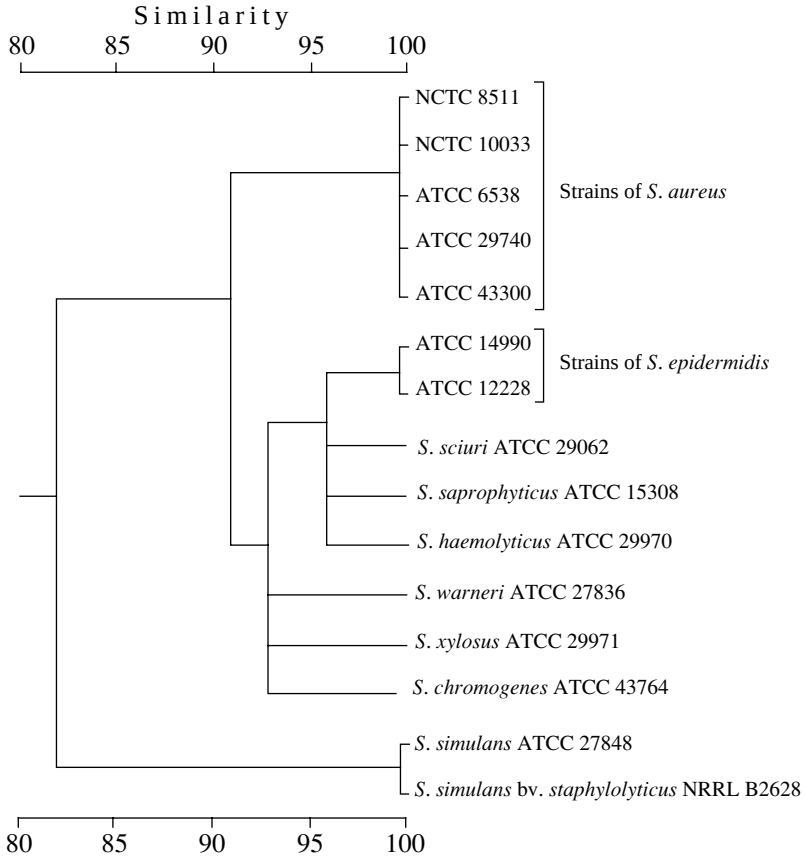

Fig. 3. Dendrogram based on the analysis of SDS–PAGE profiles of extracellular proteins of *Staphylococcus aureus* and other *Staphylococcus* strains by the unweighted pair group method with an arithmetic averages algorithm (UPGMA).

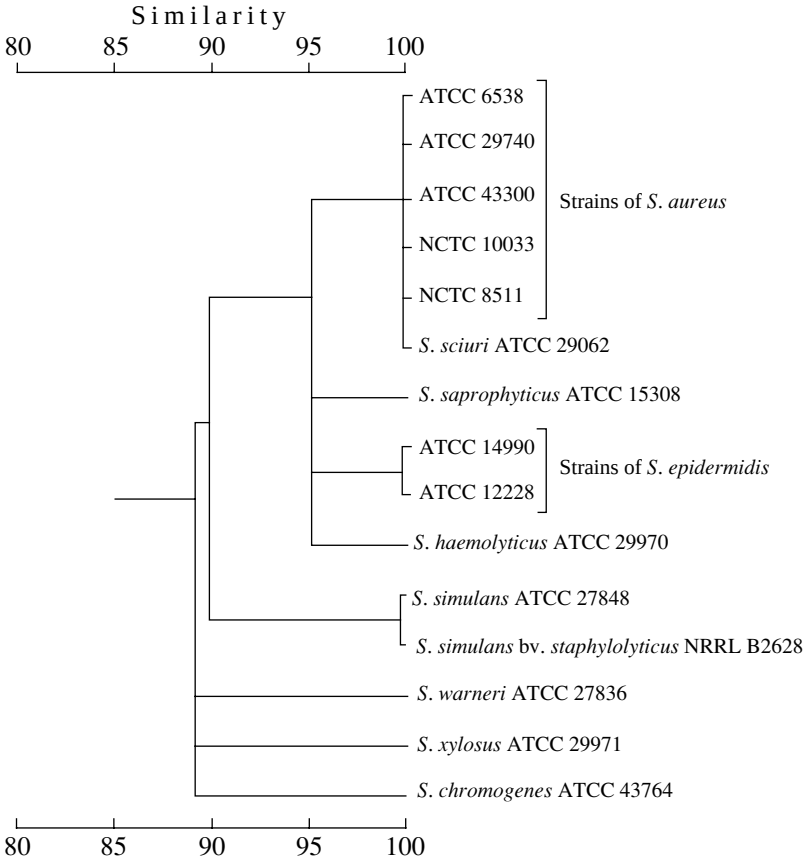

Fig. 4. Dendrogram based on the analysis of SDS–PAGE profiles of whole-cell proteins of *Staphylococcus aureus* and other *Staphylococcus* strains by the unweighted pair group method with an arithmetic averages algorithm (UPGMA).

Cluster dendrograms produced by numerical analysis of whole-cell protein profiles and extracellular protein profiles using the unweighted pair group method with an arithmetic averages algorithm are shown in Figs. 3 and 4. It should be noted that all of the staphylococcal strains tested clustered according to their biovar type. As seen from Figs. 3 and 4, the representatives of *S. aureus* formed an electrophoretically homogeneous cluster with a 100% similarity level. The same was true for *S. epidermidis* and *S. simulans* strains. Other strains tested formed single-member clusters with a similarity of about 90% with other clusters. A good correlation was found between the two dendrograms of electrophoretic protein clusters. Strains of particular *Staphylococcus* species were recovered in discrete clusters both when whole-cell and when extracellular protein profiles were analyzed quantitatively.

Our results obviously show that the electrophoretic method can provide valuable epidemiological information and may be used in the isolation of *Staphylococcus* strains. Some other researchers [8, 17, 19, 24] obtained similar results. At the same time, it has been reported that SDS–PAGE of polypeptides of whole-cell extracts does not readily provide data for the establishment of typing schemes for staphylococci [11, 20]. Our results are in agreement with the findings of Clink and Pennington [21], who reported that the protein patterns of whole cells of *S. aureus* strains and other species of staphylococci are similar, although minor differences occur. Our findings are also in coherence with the results of Krikler *et al.* [25], who observed that *S. aureus* strains exhibit slight band variation in their SDS–PAGE whole-cell protein profiles.

We used the MINITAB program to analyze the data because of difficulties in the visual interpretation of the bands obtained in SDS–PAGE of whole-cell proteins and extracellular proteins. The whole-cell protein profiles were more complex and less distinguishable than the profiles of extracellular proteins. According to our results, both procedures were insufficient to differentiate the strains of *S. aureus* and other *Staphylococcus* strains at either the species or the subspecies level. It was reported that the profiles of whole-cell and extracellular proteins allow species differentiation for most bacterial genera, but not differentiation of subspecies [19, 26]. However, Atalan [16] differentiated strains of *Streptomyces* using SDS–PAGE of whole-cell proteins and Cokmus and Yousten [15] differentiated strains of *Bacillus sphaericus* using this method. Some research-

ers [11, 12, 14, 26] have also used these methods to identify and characterize strains of some bacterial genera.

Protein electrophoresis has been of great value for the delineation of numerous bacterial taxa [13]. Each of the different electrophoretic techniques has its own discrimination level and field of application. It is widely acknowledged that the electrophoretic separation of cellular proteins is a sensitive technique that may provide information on the similarity of strains at and below the species level. In addition, it is also generally accepted that the comparison of electrophoretic protein patterns provides a reliable measure of genomic relatedness. Given this context, it is very encouraging that, upon quantitative analysis of whole-cell and extracellular protein profiles, the strains of *Staphylococcus* spp. formed clusters with at least 90% similarity.

To conclude, an appropriate electrophoretic method of protein analysis should be applied taking into account the purpose of this analysis and the taxonomic levels at which the bacteria are to be characterized and identified. SDS–PAGE of extracted whole-cell and extracellular proteins is a relatively rapid and simple method that can be easily standardized.

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