A Comparative Study of Bacteriophage, Serological and Enzymic Typing of Staphylococcus aureus

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ABSTRACT

The presently accepted method for marking individual strains of Staphylococcus aureus for epidemiological investigation is bacteriophage typing. However, phage typing is not a stable marker and many strains cannot be successfully typed. Serological typing is not readily available and preparation of sera is difficult. The aminopeptidase profile method described by Krawezyk and Huber5 was used to mark strains of S. aureus. Profiles were constructed diagramming the percent hydrolysis of 22 \( \beta \)-naphthylamide substrates by 15 isolates of S. aureus. The aminopeptidase profiles (APP) were thought to be more complete in marking individual strains of S. aureus when compared to bacteriophage and serological typing. Thusly, this method has applicability in the clinical laboratory for epidemiological investigation of S. aureus.

Introduction

Bacterial infections produced by staphylococci continue to be encountered in high frequency in medical practice. The presently accepted method for marking individual strains of Staphylococcus aureus for epidemiological investigation is bacteriophage typing. However, phage typing is not a stable marker and can change through interaction with wild phages, lysogeny, transduction or loss of prophage. Additionally, many strains cannot be successfully phage typed.4,8,10

An alternative method of typing S. aureus is the serotyping system devised by Oeding.7 However, successful serotyping is dependent upon properly prepared antisera, and the lack of availability of antisera presents a definite problem.

In 1967, Westley et al9 proposed the use of aminopeptidase activity as a means of bacterial identification. Since then, numerous publications have revealed the advantage of this method3,6 Therefore, aminopeptidase profiles (APP) were performed on various isolates of S. aureus in an attempt to determine whether or not there are different patterns for different phage types and serotypes and, possibly, specific isolates within the same type, thereby providing an additional epidemiologically useful tool.
Materials and Methods

Twenty five epidemiological cultures of *Staphylococcus aureus* were kindly supplied to the authors and the cultures were phage typed by the supplier. Three cultures were of phage type 94/96, five others were of phage type NT/1136 and the last set of three cultures were of phage type 95. Three cultures were of phage type 29/52/52A+/80+, four others were phage type 3A/3C/55/71 and the last set of three cultures were phage type 83A/85. Four isolates were not typable either by the international or by experimental typing using routine test dilution (RTD). The phage typed isolates were serotyped according to the method of Oeding.

Aminopeptidase profiles of the cultures representing the 25 different isolates of *S. aureus* were determined by the method of Krawczyk and Huber. Bacteria were grown on Trypticase Soy Agar (TSA) slants for 24 hours and then dispersed in 0.05 M tris-HCl buffer, pH 8.0. The concentration of bacteria was standardized spectrophotometrically at 420 nm to nephelometer tube No. 1 (3.0 × 10⁸ bacteria/ml), and 0.1 ml of bacterial suspension was dispersed into 1 ml of 22 different beta-naphthylamides (table I). Hydrolysis of the beta-naphthylamides to the free amino acid and beta-naphthylamine was determined after incubation for 24 hours at 37°C, by measuring the fluorescence of the beta-naphthylamine released from the beta-naphthylamides, using an Amino-fluorocolorimeter with a Corning 7-60 narrow band pass primary filter (365 nm) and a Wratten 47-B narrow band pass secondary filter (415 nm).

The beta-naphthylamides (2 × 10⁻⁵M) were dissolved in 0.05 M tris-HCl buffer, pH 8.0, and refrigerated until used. Beta-naphthylamine and tris-HCl buffer were also assayed to determine the maximum fluorescence obtainable with complete hydrolysis and the background fluorescence, respectively. The fluorescence of solutions of individual naphthylamides that were not inoculated with bacteria was also measured and subtracted from the readings obtained, to correct for background fluorescence, and profiles were constructed diagramming the percent of hydrolysis of each naphthylamide by the tested bacteria. Ten replicates were used for each isolate, and data are presented as the average percent of hydrolysis by isolates.

Results

Fourteen different aminopeptidase profiles (APP) were obtained based on decreasing order of hydrolysis of the eight most active Beta-naphthylamides (table II). Although a total of 22 β-naphthylamides were used, the remaining 14 were excluded as the hydrolysis was either too low or there were no significant differences among the strains. All reported values are true values (i.e., base values for substrates were subtracted). The precision and reproducibility of data were excellent as the standard error of the mean for the hydrolysis of each amide within each profile ranged from a low of ± 0.76 percent to a high of ± 3.02 percent. Differences greater than this are characteristic of strain difference.

The overall aminopeptidase activity of *S. aureus* appeared to be low as none of the substrates was hydrolyzed 100 percent after 24 hours of incubation. This is probably due to the enzyme activity of the *S. aureus*, since many other organisms were found to hydrolyze some of the substrates 100 percent after 24 hours. The 24 hours reading was used since the enzyme activity of *S. aureus* was shown to be maximal after 24 hours. The 24 hours reading was used since this was the accepted standard procedure of the method and it was shown that further incubation would yield nonreproducible aminopeptidase profiles. Nevertheless, the present study demonstrates that some phage types...
had distinctive profiles. The three strains representing phage type 29/52/52A/80+ fit aminopeptidase profile identified as APP I. The four strains representing phage type 3A/3C/55/71 had two different profiles identified as APP II and III. Phage type 83A/85 also had two different aminopeptidase profiles (IV and V). All three strains representing phage pattern 94/96 fit profile VI. One of the three strains representing phage type 95 had an aminopeptidase profile (VII) whereas two other strains had a different profile (VIII). The five strains representing phage pattern NT/1136 had three distinctive profiles (IX, X, XI). The four not phage typable strains also had three distinctive profiles (XII, XIII, XIV).

The results of the aminopeptidase profiles indicated that all staphylococci hydrolyzed alanyl-, methionyl-, cysteinyl- and glycyl-beta-naphthylamide to a moderate degree and glutamyl-beta-naphthylamide to the highest degree. Of all the substrates used, only valyl-beta-naphthylamide was not hydrolyzed at all.

TABLE II

Aminopeptidase Profiles (APP) of Staphylococcus aureus Isolates by Decreasing Order of Percent Hydrolysis ± Standard Error of Mean of Beta-naphthylamides

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>APP</th>
<th>Beta-naphthylamides*</th>
</tr>
</thead>
<tbody>
<tr>
<td>3749</td>
<td>GLU</td>
<td>48 ± 1.74</td>
</tr>
<tr>
<td></td>
<td>ALA</td>
<td>33 ± 1.31</td>
</tr>
<tr>
<td></td>
<td>CYS</td>
<td>31 ± 1.28</td>
</tr>
<tr>
<td></td>
<td>GLY</td>
<td>28 ± 1.32</td>
</tr>
<tr>
<td></td>
<td>MET</td>
<td>27 ± 1.40</td>
</tr>
<tr>
<td></td>
<td>ARG</td>
<td>18 ± 0.81</td>
</tr>
<tr>
<td></td>
<td>ASP</td>
<td>17 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>LYS</td>
<td>10 ± 0.77</td>
</tr>
<tr>
<td>3946</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>73 ± 2.86</td>
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<tr>
<td></td>
<td></td>
<td>33 ± 1.31</td>
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<td>31 ± 1.28</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>10 ± 0.77</td>
</tr>
<tr>
<td>5206</td>
<td>GLU</td>
<td>48 ± 1.14</td>
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<tr>
<td></td>
<td>ALA</td>
<td>43 ± 1.64</td>
</tr>
<tr>
<td></td>
<td>CYS</td>
<td>30 ± 1.02</td>
</tr>
<tr>
<td></td>
<td>GLY</td>
<td>25 ± 1.03</td>
</tr>
<tr>
<td></td>
<td>MET</td>
<td>23 ± 1.22</td>
</tr>
<tr>
<td></td>
<td>ARG</td>
<td>13 ± 0.79</td>
</tr>
<tr>
<td></td>
<td>ASP</td>
<td>11 ± 0.85</td>
</tr>
<tr>
<td></td>
<td>LYS</td>
<td>7 ± 0.78</td>
</tr>
<tr>
<td>3926</td>
<td>GLU</td>
<td>48 ± 1.14</td>
</tr>
<tr>
<td></td>
<td>ALA</td>
<td>43 ± 1.64</td>
</tr>
<tr>
<td></td>
<td>CYS</td>
<td>30 ± 1.02</td>
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<tr>
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<tr>
<td></td>
<td>LYS</td>
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</tr>
<tr>
<td>5341</td>
<td>GLU</td>
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<td></td>
<td>ALA</td>
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<tr>
<td></td>
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<td></td>
<td>LYS</td>
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<tr>
<td>5338</td>
<td>GLU</td>
<td>48 ± 1.14</td>
</tr>
<tr>
<td></td>
<td>ALA</td>
<td>43 ± 1.64</td>
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<td>LYS</td>
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</tr>
</tbody>
</table>

*See table I for full name of beta-naphthylamides.
While different serotypes were found in five of the six phage sets (A, B, C, D, F) of S. aureus, different aminopeptidase profiles were found in four of the six phage sets (B, C, E, F) (table III). These profiles correlated with serotype better than with phage typing, and both serotypes and aminopeptidase profiles were more specific in marking an individual strain within a phage type.

Discussion

Aminopeptidases are, by gross definition, proteolytic enzymes capable of hydrolyzing an aminopeptidase linkage and, specifically, the peptide linkage associated with an amino group. Since there are other proteolytic enzymes which may also have aminopeptidase activity, its measurement relates to the total activity of this enzyme. The technique which has been previously applied to the identification of plant pathogenic fungi and to differentiate the yeast phase of human pathogenic fungi and of certain bacteria was applied to Staphylococcus aureus in this study.

Fourteen different aminopeptidase profiles of S. aureus were established on the basis of the decreasing order of hydrolysis of the amino acid-beta-naphthylamides and in the different quantity of hydrolysis within the phage types. From this order, the relatedness of these organisms becomes apparent. The profiles within each bacteriophage type are more related to each other than they are to different phage groups, although all S. aureus have in common the capacity to hydrolyze glutamyl-, methionyl-, cysteinyl- and alanyl-beta-naphthylamides.

Cohen and Smith² reported that certain phage groups were somewhat heterogeneous serologically. Our data support this finding and show that certain phage groups can be further separated by both serotyping and aminopeptidase profiles. A review of table III, which compares the use of all three methods on six phage sets, reveals that phage sets A and D show good correlation among phage type, serotype and aminopeptidase type. There is only a slight serotype difference of isolate (3749) with set A and (3815) within set D. Sets B and C show differences in APP and serotype when compared to the phage pattern. Here both APP and serotype support each other. In addition, the APP profile of isolate 4109 was determined in spite of no reaction by serotyping. Set E shows similarity with all three typing systems except for APP of isolate 3765. It appears that APP is more specific than serotype in this case. Set F shows three distinct APP's for the same phage type and two different serotypes. The APP proved again to be more sensitive.

Both of the typing methods have their advantages and disadvantages. Phage typing seems to be highly specific for certain strains of S. aureus, but less specific for others. A definite disadvantage is that...
the phage pattern may change through interaction with wild phage, lysogeny, transduction or loss of prophage. In addition, there are strains of *S. aureus* which are not phage typable.

Serotyping is apparently more specific than phage typing, but its usefulness is dependent upon the preparation and the quality of the antisera (which is not always monovalent). It is also difficult to find centers which can routinely serotype strains.

Aminopeptidase profiles, which in some cases clearly showed more specificity than either of the previously mentioned methods, provide new acceptable means for marking strains of *S. aureus*, even when these strains are not phage typable,—thus becoming a useful additional method for epidemiological studies. Successful aminopeptidase profiles require meticulous attention to details such as inoculum size, incubation time and temperature, as well as substrate concentration. Although the environmental constraints imposed by these requirements should not normally pose insurmountable problems in the average clinical laboratory, the utmost of care is, nevertheless, necessary to assure reliability and reproducibility. It appears that, of the three typing methods studied, APP could have the greatest of applicability in the clinical laboratory since it relies on a fluorocolorimeter and on chemical reagents.

References

5. **Krawczyk, K. and Huber, D. M.:** Standardization of aminopeptidase profiles for identification of plant pathogens. Purdue University Agricultural Experiment Station Journal, No. 4975, 1974.