INTRASPECIFIC AND INTERSPECIFIC TRANSFORMATION IN STREPTOCOCCI

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ABSTRACT

Perry, Dennis (Northwestern University Medical School, Chicago, Ill.), and Hutton D. Slade. Interspecific and interspecies transformation in streptococci. J. Bacteriol. 88:595-601. 1964.—Interspecific transformation reactions, employing streptomycin resistance as a marker, were performed with eight strains of streptococci belonging to three serological groups (F, H, and O) and one ungroupable (UG) strain. Generally, autotransformation (within the same strain) was most efficient. Homotransformation (between different strains of the same serological group), however, was sometimes as efficient or slightly better. Heterotransformation (between different serological groups) yielded the least number of transformants or none at all. The rate of transformation of different strains varied from $2.0 \times 10^{-7}$ to $7.6 \times 10^{-4}$. Group H streptococci exhibited the highest rate of autotransformation, followed by groups F and O, and strain UG. The results of heterotransformation reactions revealed that a genetic relationship exists between various strains of different serological groups. No transformation, however, occurred between F and O strains, indicating a lack of genetic homology between these serological groups of streptococci. Deoxyribonucleic acid (DNA), isolated by physical and enzymatic methods from a group O and a UG strain, failed to induce transformation. DNA from these strains, however, significantly inhibited the transforming ability of other DNA preparations. Heat and mechanical shear resulted in a marked decrease in this inhibitory property.

Investigations have shown that genetic transformation can occur between microorganisms of different species (for review, see Marmur, Falkow, and Mandel, 1963). The efficiency of interspecific transformation, however, is usually considerably less than that of intraspecific transformation. Schaeffer (1958) hypothesized that the lower efficiency of interspecific transformation is due to a lack of genetic homology between donor and recipient deoxyribonucleic acid (DNA) molecules. In addition, several investigators have been able to correlative similarity in DNA base composition with ability to undergo interspecific transformation (Schaeffer, Edgar, and Rolfe, 1960; Catlin and Cunningham, 1961; Marmur, Seaman, and Levine, 1963). These observations suggest that transformation may be of considerable taxonomic value in studies on microbial classification.

Studies on the interspecific transformation of streptococci have been limited to a few strains. Initially, Bracco et al. (1957) reported the transformation of two serologically ungroupable strains of streptococci with DNA from homologous and heterologous ungroupable strains and also with pneumococcal DNA. Pakula, Hulanicka, and Walczak (1959) confirmed the work of Bracco et al. (1957) and also reported the transformation of group H streptococci by streptococcal, pneumococcal, and staphylococcal DNA. The transformation of streptococci by staphylococcal DNA was nonreciprocal and needs further investigation. Reciprocal transformation reactions between Streptococcus and pneumococcus, however, indicate that a close relationship exists between these genera (Ravin and De Sa, 1964). Pakula (1961) showed that some streptococci seem more closely related to the pneumococcus than to other streptococci.

These investigations were extended by Perry and Slade (1962) to include the transformation of groups F and O streptococci. It was shown that DNA from different serological groups of streptococci produced different levels of transformation, and that some strains exhibited a higher level of transformation than others, regardless of the source of DNA. In addition, observations in our laboratory indicate that optimal conditions for transformation of various strains of streptococci may differ and thereby
influence the rate of transformation (Perry and Slade, 1963).

Of the 17 serological groups of streptococci, only three groups (F, H, and O) and several ungroupable strains have been transformed (Perry and Slade, 1962). The purpose of these investigations was to examine the genetic relationship between the transformable streptococci, with particular emphasis on correlating serological classification with efficiency of transformation. While unrelatedness cannot be assured by the failure to demonstrate transformation, it was felt that the results obtained might be significant in determining the lack of transformation in other serological groups of streptococci. For simplicity, the following terms as described by Bracco et al. (1957) were employed in describing the various types of transformation reactions among the streptococci: autotransformation (within the same strain), homotransformation (between different strains of the same serological group), and heterotransformation (between different serological groups).

**Materials and Methods**

**Bacterial strains.** The following eight strains of streptococci comprising three serological groups and one ungroupable (UG) strain were employed in these investigations: group F, strains C628, 8RS76, and H127; group H, strains Challis and SBE; group O, strains B357 and B486; UG, strain MD. Groups F and O streptococci were obtained from R. Lancefield, and group H strains were obtained from R. Pakula. The UG strain was isolated in our laboratory. The serological classification of all strains was carefully checked by the precipitin test (Swift, Wilson, and Lancefield, 1943).

Streptomycin-resistant mutants were obtained from all strains as previously described, and were designated with the suffix SR (Perry and Slade, 1962). Each strain was resistant to over 200 μg of streptomycin per ml.

**Preparation of DNA.** DNA was isolated from some strains of streptomycin-resistant mutants by lysis with *Streptomyces albus* enzyme according to procedures described previously (Perry and Slade, 1962). DNA from a majority of the strains, however, was isolated by rupture of the cells with a French or Sagers (1962) press at a pressure of 13,000 to 15,000 psi. The presses were stored at -20 C for 24 hr prior to use. Cells for the French press were suspended to 10% (wet weight) in 0.15 M NaCl plus 0.015 M sodium citrate (SSC) containing 2% sodium lauryl sulfate before use. Cells for the Sagers press were packed by centrifugation and frozen before use. After crushing, the frozen mass of broken cells from the Sagers press was then suspended to 10% in SSC. DNA was deproteinized by the method of Sevag, Lackman, and Smolens (1938) or that of Young and Spizizen (1961). The quantitative determination of DNA was carried out by the diphenylamine method (Dische, 1955). The sterilization of DNA was performed according to procedures described previously (Perry and Slade, 1962). Stock solutions of DNA were stored at 4 C in SSC.

**Transformation procedure.** Transformation reactions were performed as described previously (Perry and Slade, 1962, 1963). Transforming DNA was employed at a concentration of 5 μg/ml. Appropriate dilutions of streptococci were spread onto sheep blood-agar containing 100 μg of streptomycin per ml for the detection of transformants.

**DNA inhibition experiments.** Various dilutions of inhibiting DNA (0.001 to 5.0 μg/ml) and 0.1 μg of transforming DNA (Challis-SR) were added simultaneously to recipient cells (group H, strain Challis). The remainder of the procedure was as described above.

Inhibiting DNA was denatured by heating or mechanical shearing. In the heating experiments, 0.5-ml volumes of DNA (100 μg/ml) in screw-capped tubes were heated in a water bath at 100 C for 10 min. The tubes were then quickly plunged into an ice bath. DNA was sheared by aspirating 0.5 ml (100 μg/ml) 20 times through a 24-gauge needle with a 1-ml syringe. These DNA preparations were tested immediately for their ability to inhibit transformation. The activity of transforming DNA remaining after mixing with inhibiting DNA was defined as the per cent residual activity. The activity of transforming DNA alone was assigned a value of 100% for a basis of comparison.

**Results**

The conditions under which the transformation reactions were performed were identical, except for differences in time for the appearance of competence. Perry and Slade (1963) showed previously that the period of optimal competence
of various strains of streptococci is attained at different times. The time for the appearance of competence of the eight strains of streptococci employed in these investigations was as follows: 1 hr for group H, strain Challis, group F, strains C628 and H127, and UG, strain MD; 2 hr for group H, strain SBE, group F, strain 8RS76, and group O, strain B357; and 3 hr for group O, strain B486. In addition, the concentration of recipient cells influenced the rate of transformation and varied depending on the strain employed. Generally, the concentration of cells varied from $10^4$ to $10^7$ per ml.

Table 1 shows the results obtained when various strains of streptococci were exposed to different preparations of DNA. In general, autotransformation was most efficient, although homotransformation, in certain instances, was as efficient or slightly better. The transformation of group H, strain SBE, with Challis-SR DNA was almost one and one-half times greater than the transformation of this strain with SBE-SR DNA. Other examples in which homotransformation was equal to or greater than autotransformation were observed in the following experiments: group F, strains C628 and H127 x 8RS76-SR DNA; group F, strain 8RS76 x H127-SR DNA; and group O, strain B486 x B357-SR DNA. As was to be expected, heterotransformation yielded the lowest number of transformants. In one instance, the heterotransformation of group F, strain C628, with SBE-SR DNA was more efficient than was the auto- and homotransformation of this strain. It can be seen, however, that the rate of transformation of group F, strain C628, was not significantly influenced by the source of DNA. That is, the rate only varied from $0.16 \times 10^{-4}$ to $0.91 \times 10^{-4}$, regardless of the source of the DNA. The transforming activity of C628-SR DNA was considerably less than that of H127-SR and 8RS76-SR DNA in most cases (Table 1). Whether this result is due to genetic or physical factors is unknown at the present time.

Group F, strain 8RS76, was not transformed with SBE-SR and Challis-SR DNA. This result was unexpected, since the other group F strains (C628 and H127) were transformed by DNA from both group H streptococcal strains (Challis-SR and SBE-SR). No heterotransformation occurred between groups F and O streptococci. Group H streptococci possessed the highest

<table>
<thead>
<tr>
<th>DNA recipient</th>
<th>SBE (H)</th>
<th>Challis (H)</th>
<th>C628 (F)</th>
<th>H127 (F)</th>
<th>8RS76 (F)</th>
<th>B357 (O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBE (H)†</td>
<td>35</td>
<td>51</td>
<td>0.07</td>
<td>2.8</td>
<td>2.1</td>
<td>0.83</td>
</tr>
<tr>
<td>Challis (H)</td>
<td>50</td>
<td>76</td>
<td>0.52</td>
<td>5.6</td>
<td>3.0</td>
<td>3.3</td>
</tr>
<tr>
<td>C628 (F)</td>
<td>0.71</td>
<td>0.16/0.50</td>
<td>0.27</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>H127 (F)</td>
<td>0.34</td>
<td>0.61/0.73</td>
<td>15</td>
<td>15</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>8RS76 (F)</td>
<td>—</td>
<td>0.24</td>
<td>5.1</td>
<td>3.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B357 (O)</td>
<td>0.02</td>
<td>0.04</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B486 (O)</td>
<td>0.04</td>
<td>0.04</td>
<td>—</td>
<td>—</td>
<td>0.19</td>
<td>—</td>
</tr>
<tr>
<td>MD (UG)‡</td>
<td>0.01</td>
<td>0.02/0.003</td>
<td>0.003</td>
<td>0.002</td>
<td>0.05</td>
<td>—</td>
</tr>
</tbody>
</table>

* Rate of transformation is defined as the ratio of transformants to total colony centers ($\times 10^{-4}$); — indicates no transformation.
† Letters in parentheses after strain number represent serological group.
‡ Refers to ungroupable streptococci.

The results in Fig. 1 indicate that DNA from MD-SR and B486-SR prepared by S. albus enzyme and a Sagers press, respectively, inhibited the transformation of group H, strain Challis, to a degree comparable to DNA prepared from a Challis streptomyacin-sensitive strain. In this, and succeeding experiments, transforming DNA was employed at a concentration of 0.1 µg/ml. It was observed that, at a ten-fold excess concentration of inhibiting DNA over transforming DNA, only 16 to 24% residual activity remained. An increase in the concentration of inhibiting DNA resulted in a further drop in the residual activity. It was observed, however, that when concentrations of inhibiting DNA between $10^{-3}$ to $10^{-4}$ µg/ml were used the degree of inhibition was not as significant.
FIG. 1. Effect of nontransforming DNA on the transforming ability of DNA from strain Challis-SR. Transforming DNA was employed at a concentration of 0.1 μg/ml.

TABLE 2. Effect of heat on the inhibiting ability of nontransforming deoxyribonucleic acid

<table>
<thead>
<tr>
<th>Inhibiting DNA (μg/ml)</th>
<th>Per cent residual activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unheated</td>
</tr>
<tr>
<td>MD-SR (UG), <em>Streptomyces albus</em>†</td>
<td>12.1</td>
</tr>
<tr>
<td>MD-SR (UG), French press</td>
<td>36.7</td>
</tr>
<tr>
<td>B486-SR (O), Sagers press</td>
<td>13.3</td>
</tr>
<tr>
<td>B486-SR (O), French press</td>
<td>52.7</td>
</tr>
<tr>
<td>Challis (H), Sagers press</td>
<td>8.8</td>
</tr>
<tr>
<td>Challis-SR (H), French press‡</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* DNA was denatured by heating 0.5 ml (100 μg/ml) at 100 C for 10 min in screw-capped tubes. Tubes were placed in an ice bath immediately after heating.
† Transforms to method of DNA extraction.
‡ Transforming DNA (Challis-SR) was used at a concentration of 0.1 μg/ml. The recipient in each experiment was strain Challis.

The effect of heat on the inhibiting ability of MD-SR and B486-SR DNA is shown in Table 2. Results showed that over 90% of the residual activity of transforming DNA was regained after heating inhibiting DNA at 100 C for 10 min. When Challis DNA (streptomycin-sensitive and homologous for DNA-recipient strain) was heated, only 53.2% residual activity was regained. The heating of transforming DNA (Challis-SR) resulted in a decrease in activity to less than 1%. It was interesting that MD-SR and B486-SR DNA prepared by the French press method were much less inhibitory than the DNA preparations prepared by the Sagers press and *S. albus* enzyme methods.

The effect of mechanical shearing on the inhibiting ability of MD-SR and B486-SR DNA also was examined (Table 3). It was evident that shearing decreased the inhibiting ability of these DNA preparations. The effect of shearing was somewhat greater on Challis DNA. Approximately 80% of the transforming activity of Challis-SR DNA was destroyed upon shearing.

TABLE 3. Effect of mechanical shear on the inhibiting ability of nontransforming deoxyribonucleic acid

<table>
<thead>
<tr>
<th>Inhibiting DNA (μg/ml)</th>
<th>Per cent residual activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
</tr>
<tr>
<td>Challis (H), Sagers press†</td>
<td>8.8</td>
</tr>
<tr>
<td>MD-SR (UG), <em>Streptomyces albus</em>‡</td>
<td>8.8</td>
</tr>
<tr>
<td>B486-SR (O), Sagers press</td>
<td>12.9</td>
</tr>
<tr>
<td>Challis-SR (H), French press‡</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* DNA was sheared by aspirating 0.5 ml (100 μg/ml) 20 times through a 24-gauge needle with a 1-ml syringe.
† Refers to method of DNA extraction.
‡ Transforming DNA (Challis-SR) was used at a concentration of 0.1 μg/ml. The recipient in each experiment was strain Challis.

DISCUSSION

Many instances of nonreciprocal transformation have been observed within the genus *Streptococcus*. For example, DNA from most of the 17 serological groups of streptococci will transform group H, strain Challis, and yet, these strains are practically all nontransformable (Perry and Slade, 1962). Pakula et al. (1969) also showed that nonreciprocal transformation occurred between a group H streptococcus and a staphylococcus.

It was demonstrated by Perry and Slade (1962) that several strains of nontransformable strepto-
coccii incorporated significant amounts of P32-labeled DNA. The inability of these strains to transform was not due to a lack of DNA penetration. Schaeffer (1957) also found that the lower frequency of interspecific transformation in *Haemophilus* was not due to a lack of DNA adsorption or penetration. P32-labeled DNA was taken up in equal amounts by different species. Mention has been made already of the suggestion by Schaeffer (1958) that the low frequency of interspecific transformation was due to incomplete base homology of the donor and recipient DNA molecules. Results obtained in our laboratory strongly suggest that physiological factors are probably as influential, particularly in non-reciprocal transformation reactions. In support of these observations, Krauss and MacLeod (1963) found that, in the presence of pleural fluid, a streptococcus transformed at a higher frequency than did pneumococcus with pneumococcal DNA. The autotransformation of pneumococcus was more efficient when human plasma was employed in the transforming medium.

The influence of genetic factors on interspecific transformation should not be minimized, although interpretations of such data should be made with caution. Catlin and Cunningham (1961) and Marmur et al. (1963) reported, in several instances, a greater frequency of interspecific over intraspecific transformation in *Neisseria* and *Bacillus*, respectively. Our results also illustrated that homotransformation was sometimes more efficient than autotransformation. Perhaps these observations may be partly explained by the investigations of Green (1959), who demonstrated that difficulties in the integration of certain markers can occur within a single species.

With some awareness of the influence of environmental and genetic factors on transformation, attempts were made to determine the genetic relationship of the various strains and serological groups of streptococci employed in these investigations. Homotransformation reactions among the three serological groups of streptococci revealed that the strains within each serological group are genetically related. It appears, therefore, that there is some correlation between serological classification and ability to undergo transformation.

Pakula (1961) suggested combining group H, strain Challis, and "Str. sbe, type 1H" (group H, strain SBE, in these investigations) into a single species based on results obtained in interspecific transformation studies. Our results confirmed this observation. In addition, we found that group H, strains Challis and SBE, cross-reacted quite strongly with antiserum against either of the two strains. That is, no apparent serological difference exists between these strains. It thus appears that these strains are closely related not only genetically but also serologically. Group H, strains Challis and SBE, also exhibited varying degrees of relationship to groups F and O streptococci. It might be added that five of six group H streptococci in our possession were transformable, although at different rates (Perry and Slade, 1962).

Group F, strains H127 and 8RS76, seemed to be more closely related to each other than to group F, strain C628. However, in view of the observation that the source of DNA did not significantly influence the rate of transformation of strain C628, it was felt that conditions might not have been optimal for this strain. At the present time, no explanation can be given concerning the lack of transformation of group F, strain 8RS76, by group H DNA. Heterotransformation reactions between groups O and F streptococci indicated a lack of genetic homology among some serological groups, at least along the region of the DNA molecule carrying the streptomycin marker. Evidence did indicate, however, that some genetic relationship existed between groups H and O streptococci. Strain MD (UG) showed some relationship, in decreasing order, to groups O, H, and F, respectively. It should be noticed, in this respect, that strain MD (UG) and group O exhibited the same order of relationship to groups H and F.

Efforts to obtain transformation with B486-SR and MD-SR DNA have thus far been fruitless. It is improbable that the methods of isolating these preparations are responsible for the lack of transforming ability, since DNA from other strains prepared by the same procedures, was able to cause transformation. The release of DNA from the streptococci by any of the three procedures yielded preparations which were equally efficient in transformation reactions. Partial degradation of the DNA most likely occurred.

The difficulties associated with the rupture and enzymatic lysis of the streptococci have been
a major obstacle in studies on the transformation of these microorganisms. Transforming DNA prepared from cells broken in a French or Sagers press probably underwent some degradation. The amount of degradation, however, does not significantly affect the transforming activity, since the rates of transformation in these studies are comparable to those of other investigators. Also, the melting-point curve of DNA released by a French pressure cell from a group A SR strain was normal in all respects. (We are indebted to M. Mandel for the preparation and examination of this DNA.) It also should be added that, in preliminary studies with other transforming media, it was possible to consistently obtain over 1% transformants. Ravin and De Sa (1964) observed a decrease in the frequency of transformation with DNA prepared by S. albus enzyme. Our S. albus enzyme was purified by (NH₄)₂SO₄ precipitation, and the rate of transformation by DNA from these preparations was not significantly different from those rates obtained with DNA prepared by other means.

Hotchkiss (1954) and Alexander, Leidy, and Hahn (1954) observed that transformation was inhibited by DNA not carrying the marker being scored. Inhibition was also caused by calf-thymus DNA, indicating that the receptor sites for which the DNA molecules are competing are nonspecific (Schaeffer, 1958). These observations prompted an investigation into the effects of MD-SR and B486-SR DNA on transformation, assuming that severely degraded DNA would not cause inhibition. If this assumption is valid, our results indicate that the lack of transformation by MD-SR and B486-SR DNA is not due to severe degradation. Furthermore, the inhibiting ability of these DNA preparations was significantly decreased by heat and mechanical shear. It is noteworthy that nontransforming DNA prepared in a French press caused much less inhibition than did nontransforming DNA prepared by other methods. Even so, active DNA prepared by any of the procedures employed produced similar rates of transformation. An explanation for the lack of transformation with MD-SR and B486-SR DNA awaits further investigations.

These results, nonetheless, indicate that the general conclusions made concerning the genetic relationship of the three serological groups of streptococci are indeed valid. Of more importance, however, the information obtained should prove valuable in further investigations into the lack of genetic transformation among other serological groups of streptococci.

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