Intraspecies and Interspecies Transformation Reactions in Pneumococcus and Streptococcus

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ABSTRACT The efficiency of transformation of pneumococcus and a strain of viridans streptococcus (strain D) to streptomycin resistance is influenced by the species in which the mutation to resistance occurred, as well as by the species in which the mutated gene has been replicated. Pneumococcus and streptococcus strain D transform in higher frequency with DNA that has been replicated in bacteria of the same species than with DNA from the heterologous species. However, the difference between the frequencies of interspecific and intraspecific transformation is much greater with pneumococcus as receptor than with streptococcus. In addition pneumococcus transforms in higher frequency with wholly homologous (pneumococcal) DNA than with DNA from pneumococci that have replicated the streptococcal Sm^r gene. Pneumococcus is transformed in lower frequency by wholly heterologous (streptococcal) DNA than by DNA from streptococci that have replicated the pneumococcal Sm^r gene. Streptococcus behaves similarly in that wholly homologous (streptococcal) DNA transforms it more efficiently than when the transforming fragment contains a pneumococcal moiety. Streptococcus is transformed in the same or lower frequency by wholly heterologous (pneumococcal) DNA than by DNA from pneumococci that have replicated the streptococcal Sm^r gene. When erythromycin resistance was used as genetic marker instead of streptomycin resistance, similar results were found.

INTRODUCTION

Transformation reactions between pneumococcus and streptococci of the viridans group have been described previously (1). The results of inter- and intraspecies transformations described in the present paper are similar in most respects to those that were reported earlier. However, the experimental conditions have been changed in two important respects. In the first place, the number of competent pneumococci has been increased by using human plasma in the transforming system instead of human pleural fluid as a source

of serum factors (2). Second, the number of transformed bacteria has been counted after exposure to DNA for 10 minutes instead of for 8 hours. The latter method (3) permits more accurate measurement of the transforming efficiency of different preparations of DNA on the two receptor strains. Although improved techniques have not resulted in important differences in the inferences drawn previously (1), particularly with respect to changes in transforming efficiency when DNA is replicated in a foreign host, they have yielded more quantitative results on which conclusions can be more firmly based.

TABLE I

DESIGNATION OF BACTERIAL STRAINS AND DNA PREPARATIONS

Bacterial strains	Symbols for DNA
Streptococcus	
D *Sm*^{r}—spontaneous streptomycin-resistant mutant of strain D	S(s)*
D *Sm*^{r}(p)—streptomycin-resistant mutant of strain D obtained by transformation with pneumococcal DNA	S(p)
Pneumococcus	
II-R36NC *Sm*^{r}—spontaneous streptomycin-resistant mutant of strain II-R36NC	P(p)
II-R36NC *Sm*^{r}(s)—streptomycin-resistant mutant of strain II-R36NC obtained by transformation with streptococcal DNA	P(s)

* S and s represent streptococcus; P and p, pneumococcus. The small letters refer to the strain in which the *Sm*^{r} mutation occurred; the capital letters refer to the strain in which the *Sm*^{r} factor has been replicated.

MATERIALS AND METHODS

Bacterial Strains

1. STREPTOCOCCUS D, a strain of *Streptococcus* of the viridans group. D *Sm*^{r}, a streptomycin-resistant mutant selected from strain D. D *Sm*^{r} (p), a strain of strain D resistant to streptomycin following transformation by DNA from pneumococcus strain II-R36NC *Sm*^{r}.

2. PNEUMOCOCCUS II-R36NC, an unencapsulated colonial variant derived from a strain of type II pneumococcus, II-D39S. II-R36NC *Sm*^{r}, a streptomycin-resistant mutant selected from II-R36NC. II-R36NC *Sm*^{r}(s), a strain resistant to streptomycin following transformation by DNA from streptococcus D *Sm*^{r}. The symbols used to represent DNA extracted from these strains are shown in Table I.

Preparation of Transforming Extracts

Pneumococcus and streptococcus were grown in broth as has been described (4). Pneumococci were lysed by means of sodium deoxycholate. Streptococci were disrupted

by vibration with glass beads in a Mickle disintegrator (1), or they were lysed at pH 11 following the method used by Goodgal and Herriott to lyse *Hemophilus influenzae* (5). Longer fibers of DNA were obtained from streptococcus following lysis at pH 11 than after vibration with glass beads, but the transforming characteristics of the DNA preparations were similar. After the bacteria were disrupted, DNA was extracted as described previously (1, 4).

Pneumococcal DNA was diluted in a volume of saline equal to one-tenth the volume of the culture from which it had been extracted and streptococcal DNA in one-twentieth the volume. When these preparations were diluted from 1:100 to 1:10,000 in the transforming system, the curves relating the number of bacteria transformed to the dilutions of DNA added were approximately linear. The dose-response curves found when pneumococci and streptococci were transformed by DNA preparations are discussed below.

Transformation Reactions

Overnight blood-broth cultures of bacteria were diluted in neopeptone beef heart infusion broth to approximately 10^5 bacteria per ml. The bacterial suspension was added, 1 part in 20, to neopeptone beef heart infusion broth containing 10 per cent human plasma (2). 1.8 ml of broth containing bacteria and plasma was pipetted into a series of tubes and incubated at 37°C in a water bath. After 4 hours, 0.2 ml of DNA solution was added and exactly 10 minutes later, deoxyribonuclease (3).

To count the number of bacteria transformed to resistance, the cultures were diluted in broth and samples were plated in BBL brain heart infusion agar[1] containing 1 per cent defibrinated horse blood. After 2 hours at 37°C, horse blood agar containing streptomycin was layered on top. The final concentration of streptomycin was 500 μg per ml. Colonies were counted after 48 hours' incubation. This method of scoring has been described by Rolfe and Ephrussi-Taylor (6).

RESULTS

Transformation of Pneumococcus and Streptococcus by Pneumococcal and Streptococcal DNA

DNA solutions were added in dilutions of 1:10 to 1:10,000 to streptococcus D and pneumococcus II-R36NC. Examples of these experiments are shown in Table II. The figures represent the number of transformants per milliliter. Fractions equal to the number of transformants divided by the number of bacteria exposed to each DNA are plotted against dilutions of DNA in Figs. 1 and 2.

1. TRANSFORMATION OF PNEUMOCOCCUS AND STREPTOCOCCUS BY P(p) AND P(s) Fig. 1 illustrates the transformation of pneumococcus and streptococcus by P(p) and P(s). At each dilution of DNA, more pneumococci than streptococci were transformed by P(p). The transformation of pneumococcus

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with a saturating amount of P(p) was 13 times as great as the transformation of streptococcus with a saturating amount of the same DNA. In contrast to the results obtained with DNA P(p), no difference was observed in the rate of transformation of pneumococcus and streptococcus by P(s).

2. TRANSFORMATION OF PNEUMOCOCCUS AND STREPTOCOCCUS BY S(s) AND S(p) Fig. 2 shows the transformation ratios in experiments in which S(s) and S(p) were added to pneumococcus and streptococcus. The numbers of transformants observed per milliliter are recorded in Table II. More strepto-

TABLE II

TRANSFORMATION OF STREPTOCOCCUS STRAIN D AND PNEUMOCOCCUS STRAIN II-R36NC BY PNEUMOCOCCAL AND STREPTOCOCCAL DNA

Recipient strain	Dilution	DNA preparations P(p)	P(s)	S(s)	S(p)
Streptococcus D	1:10	9.9×10^3	1.2×10^4	2.2×10^4	1.9×10^4
	1:100	6.2×10^3	8.6×10^3	1.4×10^4	4.3×10^3
	1:1000	9.2×10^2	1.6×10^3	1.9×10^3	6.8×10^2
	1:10,000	1.3×10^2	2.9×10^2	—	8.0×10^1
	Total population	3.4×10^6	1.7×10^6	2.0×10^6	1.9×10^6
Pneumococcus II-R36NC	1:10	8.4×10^4	4.8×10^3	4.9×10^1	1.8×10^2
	1:100	5.5×10^4	2.4×10^3	5.0×10^1	7.4×10^1
	1:1000	1.5×10^4	8.4×10^2	1.1×10^1	2
	1:10,000	2.0×10^3	1.0×10^2	—	0
	Total population	2.3×10^6	7.0×10^5	2.1×10^6	3.1×10^6

The figures represent the number of transformants per milliliter.

cocci than pneumococci were transformed at each dilution of S(s) and S(p). With a saturating amount of DNA S(s), the proportion of the population of streptococcus that was transformed was about 500 times that of pneumococcus. With DNA S(p), the rate of transformation of streptococcus was 170 times that of pneumococcus.

The experiments described above were not all carried out on the same day although the same preparation of DNA was added to both recipients. From experiment to experiment, the number of bacteria transformed depended on the level of competence of the particular culture of bacteria, as well as on the source of the DNA. In spite of variations in competence from day to day, the large differences noted above between the transformation of streptococcus and of pneumococcus by streptococcal DNA S(s) and S(p) and the

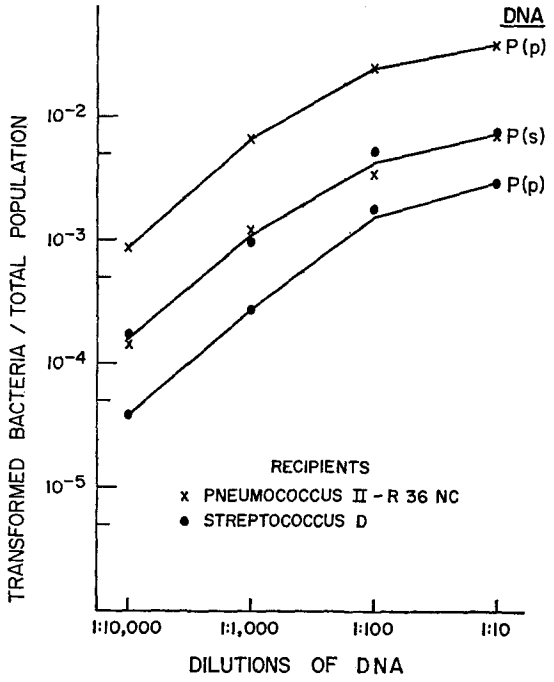

FIGURE 1. Transformation of pneumococcus and streptococcus by DNA P(p) and P(s).

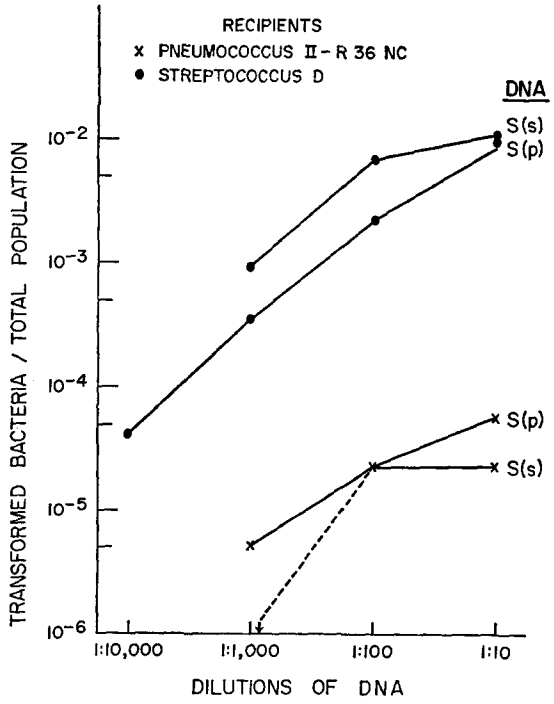

FIGURE 2. Transformation of pneumococcus and streptococcus by DNA S(s) and S(p).

smaller differences between the transformation of the two species by pneumococcal DNA P(p) and P(s) have been observed consistently.

Transformation of Bacteria of Equal Competence by Saturating Amounts of DNA

To demonstrate small differences in transformation of a strain by DNA from different sources, samples of DNA should be added to bacteria that are equally competent. Because competence may vary from one experiment to

TABLE III

TRANSFORMATION OF BACTERIA OF EQUAL COMPETENCE BY SATURATING AMOUNTS OF 4 DNA PREPARATIONS

Recipient	Experiment	DNA preparations P(p)	P(s)	S(s)	S(p)	Total population
Streptococcus D	1	2.5×10^3	5.9×10^3	2.2×10^4	1.5×10^4	1.5×10^8
	2	3.5×10^3	5.5×10^3	4.0×10^4	1.5×10^4	1.8×10^8
Pneumococcus II-R36NC	1	1.4×10^5	2.5×10^4	1.6×10^2	1.6×10^3	3.8×10^6
	2	3.5×10^4	9.7×10^3	1.8×10^2	4.4×10^2	3.4×10^6

The figures represent the number of transformants per milliliter.

TABLE IV

RATIO OF TRANSFORMANTS TO TOTAL BACTERIAL POPULATION USING SATURATING AMOUNTS OF DNA PREPARATIONS

Recipient	Experiment	DNA preparations P(p)	P(s)	S(s)	S(p)
Streptococcus D	1	1.7×10^{-3}	3.9×10^{-3}	1.5×10^{-2}	1.0×10^{-2}
	2	1.9×10^{-3}	3.1×10^{-3}	2.2×10^{-2}	8.3×10^{-3}
Pneumococcus II-R36NC	1	3.7×10^{-2}	6.6×10^{-3}	4.2×10^{-5}	4.2×10^{-4}
	2	1.0×10^{-2}	2.9×10^{-3}	5.3×10^{-5}	1.3×10^{-4}

the next, saturating amounts of DNA were added to aliquots of bacteria in the same experiment. Table III shows the number of transformants found in two of these experiments. The fractions of the populations that were transformed to streptomycin resistance are shown in Table IV. Table V shows the relative rates of transformation when the fraction for the transformation of a strain by homologous DNA is expressed as 1.

1. TRANSFORMATION OF PNEUMOCOCCUS AND STREPTOCOCCUS BY P(p) AND S(s) The numbers in Table IV show that the fraction of a pneumococcal population transformed by homologous DNA was similar to the fraction of a streptococcal population transformed by homologous DNA. For pneumo-

coccus transformed by P(p) the fractions in two experiments were 3.7×10^{-2} and 1.0×10^{-2}, and for streptococcus transformed by S(s), they were 1.5×10^{-2} and 2.2×10^{-2}.

Each strain transformed better with homologous DNA than with heterologous DNA, but the difference was much greater with pneumococcus as receptor. As shown in Table V, streptococcus transformed 10 times better with S(s) than with P(p) while pneumococcus transformed 200 to 1000 times better with P(p) than with S(s).

2. TRANSFORMATION OF PNEUMOCOCCUS AND STREPTOCOCCUS BY HETEROSPECIFIC DNA P(s) AND S(p) Experiments with heterospecific DNA P(s) and S(p) illustrate the transforming activity of DNA extracted from strains

TABLE V

RELATIVE RATES OF TRANSFORMATION OF PNEUMOCOCCUS AND STREPTOCOCCUS BY 4 DNA PREPARATIONS

Recipient	Experiment	DNA preparations			
		P(p)	P(s)	S(s)	S(p)
Streptococcus D	1	0.1	0.3	1.0*	0.7
	2	0.09	0.1	1.0*	0.4
Pneumococcus II-R36NC	1	1.0*	0.2	0.001	0.01
	2	1.0*	0.3	0.005	0.01

* The ratio of transformation of a strain by homologous DNA is expressed as 1.

that were themselves transformed interspecifically to streptomycin resistance. In these preparations, fragments of DNA from the heterologously transformed strain probably contain a copy of homologous DNA on one or both sides of a copy of the heterologous DNA.

As shown in Table V, pneumococcus transformed 2 to 10 times better with S(p) than with S(s) and 3 to 5 times better with P(p) than with P(s). Streptococcus transformed the same or up to 3 times better with P(s) than with P(p) and 1.4 to 2.5 times better with S(s) than with S(p).

Repeated experiments indicate that the differences recorded are real and that the transforming activity of DNA from heterologously transformed strains reflects the strain in which the mutation to streptomycin resistance occurred, as well as the strain in which it has been replicated. It should be noted, however, that the differences in the transformation of streptococcus and pneumococcus by P(p) and P(s) or by S(p) and S(s) are small and may not be observed if experiments are not carried out with bacteria that are equally competent.

DISCUSSION

In the present experiments, and in those reported previously (1), pneumococcus has transformed much better with pneumococcal DNA than with streptococcal DNA. However, in the previous experiments (1), more streptococci than pneumococci were found to transform with pneumococcal DNA. Under the conditions of those experiments streptococcus appeared to be a better recipient than pneumococcus for pneumococcal DNA. This was found not to be the case in the present studies in which human plasma was used in place of pleural fluid to provide the necessary serum factors in the transformation system. The number of competent pneumococci was greater with plasma than with pleural fluid and in highly competent cultures more pneumococci than streptococci are transformed by DNA from penumococcus.

In previous experiments streptococcus appeared to be transformed with equal efficiency by DNA from both species (1). In the present study, particularly when various DNA's are added to streptococci of equal competence, that is in the same experiment, more streptococci transform with streptococcal DNA than with pneumococcal DNA. However, the difference between inter- and intraspecific transformation of streptococcus strain D is much less than with pneumococcus as receptor.

Before a fragment of DNA is replicated in a species of transformable bacteria, it seems probable that it first pairs with DNA of the recipient. Pairing might be expected to happen more efficiently between DNA of the same species than between DNA of different species, and, therefore, intraspecific transformation would occur more frequently than transformation between species.

Whether bactera are exposed to DNA for 8 hours (1) or for 10 minutes as in the present study, the number of transformants appears to depend on the species in which the mutated gene for streptomycin resistance has been replicated, as well as on the species in which the mutation occurred. DNA replicated in pneumococcus transformed pneumococcus better than DNA replicated in streptococcus: P(p) and P(s) transformed pneumococcus much better than either S(s) or S(p). It appears, therefore, that the greater portion of the transforming fragment is DNA of the bacterium in which the gene has been replicated, which would result in good pairing between introduced and host DNA.

In addition, copies of the p gene in S(p) resulted in DNA that transformed pneumococcus better than S(s), and copies of the s gene in P(s) yielded DNA that transformed pneumococcus less efficiently than P(p). Therefore, the origin of the gene has some influence on the ease of replication.

Transformation reactions similar in some respects to those between pneumo-

coccus and streptococcus were found by Leidy, Hahn, and Alexander (7) and by Schaeffer (8) between *Hemophilus influenzae* and *Hemophilus parainfluenzae*. In these "species"[2] of *Hemophilus*, intraspecific transformation occurs more frequently than interspecific transformation. Likewise, introduction of DNA from one of these *Hemophilus* species into the genome of the other results in DNA that transforms the first species better than wholly heterologous DNA. However, unlike pneumococcus and streptococcus D, modification in the transforming activity of homologous DNA by the incorporation of a gene from another species into the transforming fragment has not been observed in *Hemophilus*. Since interspecies transformation in *Hemophilus* differs from that in pneumococcus and streptococcus in this respect, the hypothesis put forward by Schaeffer (8) to account for the observations in *Hemophilus* cannot be applied in detail to transformation between pneumococcus and streptococcus. It is our opinion that this difference found in our studies as compared to those of Leidy *et al.* (7) and of Schaeffer (8) may be due to the distant relationship between pneumococcus and streptococcus D, whereas *H. influenzae* and *H. parainfluenzae* are probably more closely related. It might be anticipated that matching of fragments containing transforming DNA would be less precise in distantly related microorganisms as compared to those more nearly related.

Finally, it should be noted that streptococcus strain D was found to incorporate and possibly also to replicate heterologous DNA more efficiently than pneumococcus. Thus it appears that factors in addition to the degree of similarity between the DNA of the two species must be present in order to account for the differences in transformability by heterologous DNA. Schaeffer has reported (8) that the binding of heterospecific and homospecific DNA is the same in *H. influenzae* and *H. parainfluenzae*. Whether the difference in transformation of pneumococcus and streptococcus by heterologous DNA is due to differences in uptake of heterologous DNA by these species or to intracellular events is not known.

Results similar to those described above in the transformation of pneu-

[2] The most important difference recognized between *Hemophilus influenzae* and *Hemophilus parainfluenzae* is that the former requires both hematin and a pyridine nucleotide as growth factors whereas *H. parainfluenzae* requires a pyridine nucleotide but does not require hematin. Recently Butler (9) has observed what appears to be spontaneous mutation of *H. influenzae* to *H. parainfluenzae*, that is the loss of requirement of hematin as a growth factor. Therefore, the assignment of these two representatives of the genus *Hemophilus* to different species on the basis of hematin requirement may require revision.

Pneumococcus and streptococcus strain D show many dissimilarities (1). These include differences in fermentative capacity toward at least 3 sugars, dissimilar responses both in growth and lysis in the presence of bile salts and qualitatively different sensitivity to the antimicrobial, ethylhydrocupreine (optochin). Perhaps most important of all, pneumococcus and streptococcus D do not appear to share significant surface antigens and their C carbohydrates are immunologically distinct.

mococcus and streptococcus to streptomycin resistance have also been found when resistance to erythromycin was used as genetic marker. Similarly, when an unencapsulated variant of type VIII pneumococcus (8R1) was used as a recipient the findings were the same as with strain II-R36NC. The differences observed, therefore, are not unique to streptomycin resistance and are found with more than one strain of pneumococcus.

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