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Transformation of Encapsulated *Streptococcus pneumoniae*

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We describe the high-efficiency transformation of several virulent, encapsulated isolates of *Streptococcus pneumoniae*. Transformation was effected by the induction of competence with competence factor and was apparently the result both of inducing noncompetent recipients and overcoming the inhibition imposed by the capsule.

Early studies of genetic transformation in *Streptococcus pneumoniae* demonstrated that certain unencapsulated, avirulent mutants could be transformed in vitro to the antibiotic resistance and capsular type of a donor strain with high efficiency (frequencies of greater than 1 transformant per 100 viable cells and 1 transformant per 1,000 viable cells, respectively [4, 7, 8, 10]). However, reports of the transformation of encapsulated organisms have been few (5, 14), and the efficiencies obtained were significantly lower than those for unencapsulated derivatives (less than 1 transformant per 10^6 viable cells for a fully encapsulated strain). It was suggested (6) and later shown (14) that transformation efficiency decreases with increasing capsule size. For transformation to be an effective tool in the genetic analysis of encapsulated, virulent strains, higher efficiencies are necessary. In this report, we describe the transformation of several different serotypes of encapsulated, virulent *S. pneumoniae* strains at frequencies as high as 1 transformant per 400 viable cells.

The *S. pneumoniae* strains used in these studies included Rx1 (16), a nonencapsulated and highly transformable derivative of the type 2 strain D39 (1), and DP1617 (17), a derivative of Rx1 which carries several chromosomal mutations conferring resistances to antibiotics, including streptomycin (*str-1*). The other *S. pneumoniae* strains used are described in Tables 1 and 2. V854 was kindly provided by F. Macrina, Virginia Commonwealth University, and is *Escherichia coli* DB11 harboring shuttle plasmid pVA838 (9). Crude cell lysates of *S. pneumoniae*, prepared as described by Saunders and Guild (15), were used as the source of chromosomal DNA for transformations. Purified pVA838 plasmid DNA was prepared from V854 by the method of Birnboim and Doly (2), followed by centrifugation in cesium chloride-ethidium bromide equilibrium gradients (13).

Because the development of competence in *S. pneumoniae* is dependent on cell density and growth conditions (8, 10, 18), we initially examined the ability of several encapsulated isolates to be transformed under conditions which allow for the development of peak competence in nonencapsulated strains. Cultures were grown at 37°C to a density of 3×10^8 cells per ml in Todd-Hewitt broth–0.5% yeast extract, diluted 100-fold into competence medium (Todd-Hewitt broth plus 0.5% yeast extract, 0.2% bovine serum albumin, and 0.01% $CaCl_2$), and incubated at 37°C. At 10-min intervals for 200 min, samples were removed, made 10% in glycerol, frozen in a dry ice-ethanol bath, and stored at −80°C. Competence was tested by thawing the samples at 37°C, adding 0.1 volume of DNA (approximately 1 μg/ml, final concentration), incubating at 37°C for the time specified in the legend to Fig. 1, and plating on selective medium. Chromosomal donor DNA was obtained from strain DP1617, and streptomycin (100 μg/ml)-resistant transformants were selected. Nonencapsulated strain Rx1 developed peak competence at a density of approximately 3×10^7 cells per ml (Fig. 1). However, strains WU2, DBL1, DBL5, and D39, the encapsulated parent of Rx1, were not transformed.

Competence in *S. pneumoniae* is mediated by competence factor (CF), a soluble, excreted protein that induces the transient synthesis of a group of proteins required for transformation. The addition of a competent cell culture, its supernatant, or purified CF to physiologically noncompetent cultures rapidly induces competence (11, 12, 18–20). On the basis of this, we expected that, if the capsule does interfere with transformation (6), then the presence of CF at a time when capsule production is minimal, i.e., during lag or early log phase (21), would allow for the most efficient transfor-

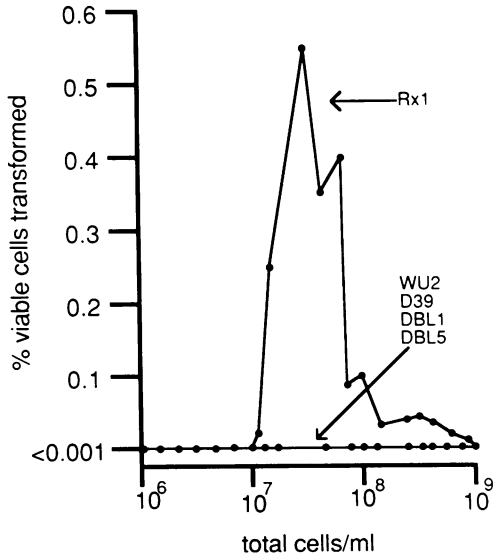

FIG. 1. Development of competence in *S. pneumoniae*. Competence was tested as described in the text. Rx1 was plated on selective (streptomycin) and nonselective medium 30 min after the addition of DNA. The encapsulated strains were plated both after 1 h and after growing to full density (ca. 10^9 cells per ml). The detectable limit was 0.001%. The encapsulated strains are described in Table 1.

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TABLE 1. Transformation of virulent[a], encapsulated *S. pneumoniae* by induction with supernatants of competent Rx1 broth cultures

Strain	Capsular type	% Viable cells transformed[b] using:		Strain source or reference
		Chromosomal DNA[c]	pVA838[d]	
DBL1	1	0.02	0.02	This laboratory[e]
D39	2	0.34	0.05	1
WU2	3	0.12	0.02	3
A66	3	<0.001[f]	<0.001[f]	1
EF3296	4	<0.001[f]	ND	C. Svanborg Eden[g]
DBL5	5	0.08	0.02	This laboratory
DBL6A	6A	<0.001[f]	ND	This laboratory
DBL14	14	0.40	ND	This laboratory
DBL23F	23F	0.004	ND	This laboratory

[a] All strains were originally isolated from human infections and have been shown to cause lethal infections in mice (1, 3; this laboratory).

[b] Cultures were plated 1 h after the addition of DNA, except for WU2, which required 4 h before transformants were observed. If no transformants were obtained, the cultures were also plated after being grown to full density (ca. 10^9 cells per ml). ND, Not done.

[c] Chromosomal donor DNA was obtained from DP1617, and Str^r transformants were selected.

[d] Erythromycin (1 μg/ml)-resistant transformants were selected. For Rx1, 0.08% of the viable cells were transformed by pVA838.

[e] DBL strains 1, 6A, 14, and 23F were obtained from B. Gray, University of Alabama, Birmingham. DBL5 was obtained from the collection of the Bureau of Biologics, Bethesda, Md., through the courtesy of J. Robbins.

[f] Below the detectable level of 0.001%.

[g] University of Goteberg, Goteberg, Sweden.

mation of these cells. We observed, however, that when mixed with competent Rx1 broth cultures, strains D39 and WU2 could be transformed both at low cell densities ($<10^3$/ml) and at densities as high as 10^8/ml, presumably because of induction by CF.

For further transformation experiments, competent broth cultures of Rx1 were prepared as described above. Because this strain releases significant quantities of CF into the surrounding medium (11), we used culture supernatants as the source of CF. Supernatants, obtained by centrifugation ($2,200 \times g$, 10 min), were heated at 60°C for 30 min to kill any remaining bacteria and were stored at −80°C. For the induction of competence, 37°C supernatants were mixed 1:1 (vol:vol) with noncompetent recipients at a density of 3×10^6 cells per ml in competence medium. The mixed cultures were incubated at 37°C for 20 min and then transformed with donor DNA as described above. With this approach, the transformation frequencies obtained for many of the virulent, encapsulated strains tested approached those of the nonencapsulated Rx1 (Table 1). The frequency of transformation was sufficiently high that we could easily obtain transformants of D39 to the capsular type of WU2 (type 3) in the absence of selection (ca. 1 transformant per 1,000 viable cells).

TABLE 2. Effect of type 3 capsule on transformation

Strain	Capsule	Cell densities examined[a]	CF added[b]	% Viable cells transformed[c]
WU2	+	1×10^6 to 2×10^9	No	<0.001[d]
WU2.M3[e]	−	2×10^6 to 5×10^9	No	<0.001[d]
WU2	+	3×10^6	Yes	0.12
WU2.M3	−	2×10^6	Yes	0.58
Rx1	−	1×10^6 to 2×10^9	No	0.55
JY2003[f]	+	1×10^6 to 1×10^9	No	<0.001[d]
JY2003	+	4×10^6	Yes	0.11

[a] Competence was tested as described for Rx1 in the text.

[b] CF was added as the supernatant of competent Rx1 broth cultures.

[c] Donor DNA was obtained from DP1617, and Str^r transformants were selected. Cultures were plated 30 min (Rx1, WU2.M3, and JY2003) or 4 h (WU2) after the addition of DNA.

[d] Below the detectable limit of 0.001%.

[e] WU2.M3 is a capsule-negative mutant derived from WU2 (this laboratory).

[f] JY2003 is Rx1 converted to capsular type 3 by transforming with WU2 DNA and backcrossing once (this laboratory).

To determine whether the inability to transform encapsulated strains in the absence of induction is the result of the presence of the capsule or of the natural incompetence of the strains, we examined the transformability of both a nonencapsulated mutant of WU2 and an Rx1 derivative transformed to capsular type 3. Even when lacking a capsule, WU2 was not transformed unless it was induced (Table 2). Thus, under the conditions used, WU2 was not naturally transformable. However, it is also apparent (Table 2) that the presence of the capsule inhibited the natural transformation of Rx1 and that the addition of exogenous CF overcame this inhibition. Interestingly, the frequencies of transformation of Rx1 and of the induced WU2 capsule-negative mutant were identical, as were the transformation frequencies of the encapsulated versions of both of these strains.

The studies described here show that many encapsulated, virulent strains of *S. pneumoniae* can be transformed if first induced to competence. The induction may be the result of inducing cells which are naturally incompetent owing to defects in the production or secretion of CF. Alternatively, CF may be produced normally but become trapped in the capsule, thus making the strain effectively incompetent. An excess amount of CF added exogenously, as in these experiments, may penetrate through the capsule and induce competence. The results described in Table 2 indicate that both mechanisms of induction are important. The inability to transform some strains even with induction may be the result of specific defects in DNA processing which cannot be overcome by the addition of exogenous CF or other factors.

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