Natural transformation of *Streptococcus crista*

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Abstract

Over the years *Streptococcus gordonii* (sanguis) Challis has become the workhorse of genetic manipulations for the sanguis group of oral streptococci. This is because strain Challis was shown in early studies to be highly naturally competent for transformation. However, Challis is not usually the most appropriate strain to use in studies which focus on oral microbial adherence. We report that other members of the newly reorganized sanguis group, particularly within the species *S. crista*, display reasonable transformation frequencies, with both plasmid and chromosomal DNA, if transformed at the appropriate time during the growth curve. The ability to transform *S. crista* may be especially important for genetic studies of biological properties that appear to be limited to these specific streptococcal strains.

Keywords: *Streptococcus crista*; *Streptococcus gordonii* Challis; Transformation; Competence

1. Introduction

A number of earlier studies indicated that oral streptococci are naturally transformable. Bracco et al. [1] showed that 2 of 16 strains of 'viridans' streptococci were naturally transformable and Perry and Slade [2] found that strains of Lancefield group H showed higher frequencies of transformation to streptomycin resistance than other serological groups. A series of studies carried out by Gaustad et al. [3] confirmed that the group H streptococci, as well as other viridans streptococci, could be transformed to streptomycin resistance. However, because the taxonomy of the oral streptococci was still evolving at the time these studies were carried out [4], it is difficult to discern the species that were being examined. In addition, very few of these transformation studies presented kinetic data and even in those studies that did, the data was usually limited to *Streptococcus sanguis* Challis (now *S. gordonii*) because of its high frequency of transformation. Indeed, this strain has become the prototype for transformation experiments among the sanguis group of oral streptococci and most laboratories use derivatives of this strain in their studies. However, this strain is not always the most appropriate model for studying streptococcal adherence or other interactions in dental plaque formation and maturation. It would be advantageous to employ other members of the sanguis group associated with these processes, as long as they are amenable to genetic manipulation. Since the taxonomy of the sanguis group of streptococci has undergone significant improvement in the last several
years [5,6], we decided to undertake more detailed studies of transformation among archetypal strains of the sanguis group frequently employed in studies of oral streptococci and dental plaque formation.

Recently, we reported that *S. cristina* CCSA, a strain isolated from dental plaque [7], is naturally competent [8]. Strain CCSA has characteristic lateral tufts of fimbriae which mediate attachment to *Fusobacterium nucleatum* and *Corynebacterium matruchotii* [9]. This interaction creates unusual 'corncob' structures in plaque. In previous reports, we used transformation: (i) to introduce a transposon into strain CC5A to isolate binding-deficient mutants [8]; and (ii) to construct strains of CC5A that carried targeted gene disruptions [10]. This report extends the usefulness of these experimental approaches by detailing the transformation characteristics of additional strains of *S. cristina* as well as those of other important members of the sanguis group.

2. Methods

2.1. Plasmid transformations

Strains were grown in 2.0 ml of brain heart infusion (BHI) broth overnight at 37°C. 10 µl of the overnight culture were diluted into 50 ml of Todd Hewitt broth (THB) that contained 10% (v/v) fetal calf serum. When the cultures reached an optical density of approx. 0.015 at 660 nm, 1 µg of the shuttle vector pDL278, (approx. 6.6 kb [8,11]) or pVA838 (approx. 9.2 kb [12]) was added to a 330 µl aliquot of cells and allowed to incubate at 37°C for 1 h. The cells were then plated, in triplicate, on BHI agar plates containing either 75 µg ml⁻¹ of spectinomycin (pDL278) or 10 µg ml⁻¹ of erythromycin (pVA838). This procedure was repeated at 45-min intervals until the streptococci were in late exponential growth. Viable cell counts were determined at each time point by plating 100 µl aliquots of cell dilutions, in triplicate, on BHI agar plates containing no antibiotic.

2.2. Chromosomal DNA transformations

1 µg of DNA, from a mutant strain of CC5A resistant to 1000 µg ml⁻¹ of streptomycin, was added to competent streptomycin-sensitive strains of the other sanguis species. Each strain was tested at its peak of competence as determined from the plasmid transformation data. Transformed cells were plated on BHI agar containing 1000 µg ml⁻¹ streptomycin. Viable cell counts were determined as described for the plasmid transformations.

2.3. Southern blotting

A representative spectinomycin-resistant colony of

![Fig. 1. Transformation of strains of *S. cristina*. The bacteria were grown as described in the text and, at 45-min intervals, a portion of the culture was mixed with 1 µg of pDL278 DNA. The mixture was incubated and plated on medium containing spectinomycin. Colony forming units (CFU) are shown as a line graph and the bars represent the number of spectinomycin-resistant transformants/ml obtained at each sampling.](image-url)
each strain was picked and grown overnight in 5.0 ml of BHI plus 75 µg ml⁻¹ of spectinomycin. Total DNA was isolated, digested with EcoRI, blotted and probed with pDL278 DNA. Hybridization conditions and probe labeling were as previously described [8]. Non-transformed CC5A DNA, digested with EcoRI, was included on the blot as a negative control.

3. Results and discussion

It was originally observed that S. cristina CC5A was naturally competent for a brief period of time [8]. This narrow window of competence not only appeared to be a characteristic of S. gordonii Challis [13] but was also found in Streptococcus pneumoniae [14]. The low frequencies of transformation reported in earlier studies of the sanguis streptococci may have been due, in part, to this short period of competency. Based on these observations, the kinetics of transformation in members of the sanguis group that have been used in adherence studies were examined. These strains are listed in Table 1.

S. cristina strains CC5A, CR3, CR311 (Fig. 1), PSH1a and PSH1b (Fig. 2) showed a narrow time interval of competence that occurred early in the exponential growth phase and that peaked between 4 and 6 h of growth. At this peak, strains PSH1a, PSH1b and CC5A had $8 \times 10^3$, $3 \times 10^2$ and $3 \times 10^2$ transformants per $10^7$ CFU, respectively (Table 1). Southern blotting was performed to demonstrate physically that transformation had occurred. All the transformants showed a single 6.6 kb hybridiza-

<table>
<thead>
<tr>
<th>Species/strain</th>
<th>No. of transformants per $10^7$ CFU</th>
<th>Source/reference</th>
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<tr>
<td></td>
<td>pDL278 DNA Strain CC5A chromosomal DNA</td>
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<td>S. cristina</td>
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<td>P. Handley [16]</td>
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<tr>
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</tr>
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<td>H. Jenkinson</td>
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</table>

*aSuperscript T denotes the type strain.
*bEach strain was transformed with 1 µg of plasmid DNA per ml as described in the text. Transformants were selected on agar medium containing 75 µg ml⁻¹ spectinomycin (pDL278) or 1000 µg ml⁻¹ streptomycin (CC5A chromosomal DNA). The background CFU were 0 for all of the strains tested with pDL278 DNA and ranged from 0 to 4 CFU/ml for all of the strains tested with CC5A chromosomal DNA. The number of transformants was determined at the sampling time that represented the maximum period of competency.
*cValues are the mean of two separate experiments. ND, not determined.
tion-positive band that corresponded to the linearized form of pDL278 (Fig. 3).

*S. sanguis* ATCC 10556 (Fig. 2), *S. gordonii* Challis 6, *S. gordonii* M5 and *S. oralis* CN3410 (Fig. 4) were also tested for transformation and each was naturally competent to various degrees. The maximum number of transformants per $10^7$ CFU ranged from $1 \times 10^5$ for strain M5 to 26 for strain ATCC 10556 (Table 1). Among the other strains tested, *S. gordonii* G9B, *S. parasanguis* FW213 and *S. oralis* ATCC 10557 were not competent under the conditions used.

The shuttle vector pVA838 also transformed *S. crista* CC5A and *S. gordonii* Challis 6. The background CFU were 0 for the strains tested. The maximum number of transformants per $10^7$ CFU was 50 and 500, respectively. These numbers were lower than those obtained with pDL278 at the peak of competency. Both vectors use the pVA380-l origin of replication in Gram-positive hosts [11,12,15].

The same set of strains were tested for the ability to take up a chromosomal marker. The results are shown in Table 1. Strains CC5A, CR3 and CR311 gave significant numbers of transformants. Strains PSH1a and PSH1b gave less than one transformant per $10^7$ CFU which we consider to be below a useful range for genetic experiments. These two strains either were not transformed by the *S. crista* DNA or recombination did not occur. Among the other strains examined, only Challis and M5 gave significant numbers of transformants. The high frequency of transformation with chromosomal DNA reported in this and other studies could explain why Challis became the workhorse for genetic experiments among the oral streptococci. We expected that the number of transformants would be significantly

![Fig. 2. Transformation of strains of *S. crista* and *S. sanguis*. Experimental conditions were the same as described in the legend to Fig. 1.](image)

![Fig. 3. Southern blot showing the presence of pDL278 in transformed strains of *S. crista*. Total DNA was obtained from each transformed strain of *S. crista*. digested with EcoRI and a Southern blot was prepared. pDL278 DNA (6.6 kb), labeled with $^{32}$P, was used as the hybridization probe. The lane marked (-)CON contained total DNA from wild-type strain CC5A.](image)
higher with plasmid versus chromosomal DNA. However, it is not clear why the opposite results were obtained with the *S. crista* strains.

We conclude that transformation among strains of *S. crista* is widespread. Many of the strains examined could be transformed with both plasmid and chromosomal DNA. This information will be useful for others wishing to employ this species in genetic studies of the biological properties associated with the development of dental plaque.

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**References**


