

Deoxyribonucleic Acid Sequence Losses in a Stable Streptococcal L Form

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A portion of the deoxyribonucleic acid sequences present in *Streptococcus faecalis* were absent in its stable L form. The remaining sequences were common to both forms.

Deoxyribonucleic acid (DNA) from parent bacteria and their derived L forms are considered very similar on the basis of their polynucleotide homologies (4, 6). However, we will present evidence that the DNA of a lysozyme-induced, stable L form derived from *Streptococcus faecalis* strain F24 is lacking some of the polynucleotide sequences present in the DNA of the parent culture. This stable L form was obtained from an original L-form colony which was replated and recloned about 30 times before revertants to colonial growth characteristic of the parent *S. faecalis* cells were no longer observed.

In addition, our data also indicate a high degree of homology of the sequences remaining in the DNA of the L form and in those of the parent culture. In contrast, preliminary reports (7) have indicated that the DNA of the stable L form was not completely homologous with that of its parent *S. faecalis* strain F24 cells.

The parent *S. faecalis* cells and the stable L-form cells, adapted to growth in liquid media, were grown for 24 hr at 37 C in Brucella Broth (Albimi Laboratories, Flushing, N.Y., supplemented with 0.43 M ammonium chloride and 0.5% glucose. Radiolabeled cells were grown in the above medium with 20 or 100 μ C of 32 P-orthophosphate per ml.

High molecular weight DNA, DNA fragments, and DNA filters were prepared, essentially, as previously described (3). In addition, cells suspended in the buffer mixture were heated to 70 C prior to lysis and extraction with chloroform-isoamyl alcohol (24:1, v/v) in the presence of 1 M NaClO₄ and 1% sodium dodecyl sulfate (5). The cells were heated prior to lysis to diminish the degradative effects of very active deoxyribonucleases. Chloroform extraction was followed by "spooling", resuspension, pancreatic ribonuclease and Pronase (Calbiochem, Los Angeles, Calif.) treat-

ments, and, finally, phenol extraction and precipitation with ethyl alcohol. Base compositions of the DNA of parent and L form were both 38 mole% guanine plus cytosine, as determined optically by their thermal transitions. *Escherichia coli* DNA (50 mole% guanine plus cytosine) was used as a reference.

Only a small proportion, about 5%, of the single-stranded parent and L-form DNA was trapped on nitrocellulose filters, as monitored spectrophotometrically at 259 nm. Therefore, it was necessary to use larger-than-anticipated amounts of input DNA to obtain the quantities (25 to 85 μ g per 20-mm filter) of trapped DNA used in this study. The poor efficiency of trapping is probably due predominantly to nuclease-related scissions of the strands in the high molecular weight DNA duplex. Small single-stranded molecules would, thus, result when the duplex is melted. Gillespie and Spiegelman (2) indicated that small molecules are less efficiently trapped than large molecules, but either large or small fixed DNA strands, once trapped, are satisfactory for polynucleotide interactions.

Figure 1 shows results of experiments done in triplicate. Radiolabeled *S. faecalis* F24 parent DNA fragments were "melted to single strands" by boiling them in 0.1 \times SSC (SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and were reacted with single-stranded parent DNA trapped on nitrocellulose filters (Fig. 1a). These reactions were achieved in the presence of systematically varied concentrations of unlabeled, single-stranded DNA fragments (competitors) prepared from parent and L-form cells. These results indicate that the L-form DNA fragments do not compete as effectively as do those from the parent in the "parent-parent" DNA reassociation reactions. Although the competitive differences were small, they were consistently observed in experiments

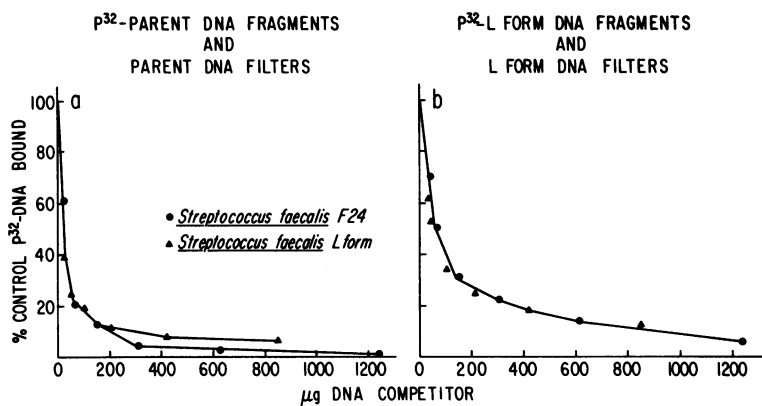


FIG. 1. Competition reactions of *S. faecalis* F24 parent and L-form DNA fragments. All reactions were done in $4 \times$ SSC plus Denhardt's preincubation mixture (1) in a volume of 0.5 ml. The reaction mixtures each contained 0.2 µg of ³²P-labeled DNA fragments which had been sheared in a French pressure cell to about 4×10^6 daltons and "melted" to single strands by boiling 5 min in $0.1 \times$ SSC. Specific activities of the fragments were 19×10^8 counts per min per µg for the parent DNA, and 36×10^8 counts per min per µg for the L-form DNA. Unlabeled, melted DNA fragments from either parent or L-form cells were added to the mixture as indicated. DNA filters (20-mm diameter) contained 35 or 84 µg of parent DNA and 25 or 40 µg of L-form DNA. Reaction mixtures were kept at 0 C until they were placed at 67 C for 36 hr. Filters were washed free of unreacted DNA by repeated washes in $4 \times$ SSC at 70 C. Blank filters retained less than 0.1% of the radioactive DNA. Without competitors, DNA filters retained 35 or 48% of the parent radiolabeled DNA fragments and 28 or 31% of the L-form fragments after incubation and washing. Results represent averages of triplicate determinations normalized to 100% binding for filters reacted without competitors.

involving two different DNA filter and competitor preparations. In no single instance were the L-form fragments as effective competitors as the parent fragments in the critical (200 to 800 µg) concentration range.

The results of the competition of unlabeled L-form or parent DNA fragments in the "L form-L form" DNA reactions are indicated in Fig. 1b. Both L-form and parent DNA fragments compete equally. The combination of the observations (Fig. 1a, b) indicates that the parent and L form contain a high proportion of common polynucleotide sequences, but that the L form lacks some of the sequences present in the parent. Direct binding experiments failed to detect the differences observed by means of competition reactions. In the L form, 4 to 6% of the sequences present in the parent (as estimated from Fig. 1a) were lacking. This situation may have arisen because of a single massive deletion or because of a series of deletions during the selection of the stable L form by cloning and independent growth. Loss of a cytoplasmic (episomal) element seems unlikely because of the extended selective process necessary to obtain the stable L form and because the missing sequences, in part, specify cell structural components. A similar, naturally occurring deletion has been described in the genus *Brucella* (3).

The extent of the observed deletion (about 100 gene equivalents if *S. faecalis* DNA is assigned 10^9 daltons) makes it likely that several different biochemical pathways were specified by the missing polynucleotide sequences. These pathways may be missing or may have altered components in the L form. Therefore, stable L-form cells exposed to parent-cell DNA should yield a spectrum of transformants of value in studies of the intermediates of cell wall biosynthesis or of other biochemical functions lost by the stable L form.

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