

## Anti-aggregation Potential of Sugars against *Streptococcus mutans* in Co-culture with *Lactobacillus rhamnosus*

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Dental caries, or cavities, are lesions which affect the greater part of the world's population. These cavities are caused by the formation of dental plaque by oral bacteria. It is known that different strains of oral bacteria are able to attach to one another and coaggregate. Research has shown that various sugars can be used to inhibit the coaggregation of these bacteria, thus preventing progression into dental caries. The dominant form of cell-cell interactions (coaggregation) occur between lectin type (protein) adhesions and oligosaccharide moieties on the bacterial cell membrane. Coaggregation can be interrupted by either denaturation of lectin or by the addition of sugar, which blocks the active lectin site [1].

One of the leading bacterial strains known to cause dental cavities is *Streptococcus mutans*, which is naturally found in the human mouth [2]. *Lactobacillus rhamnosus* is also commonly found in the human mouth and is known to coaggregate with *S. mutans*. The goal of this study was to determine the inhibitory ability of lactose, D-fructose, D-fucose, and D-galactose on the coaggregation of *S. mutans* with *L. rhamnosus*.

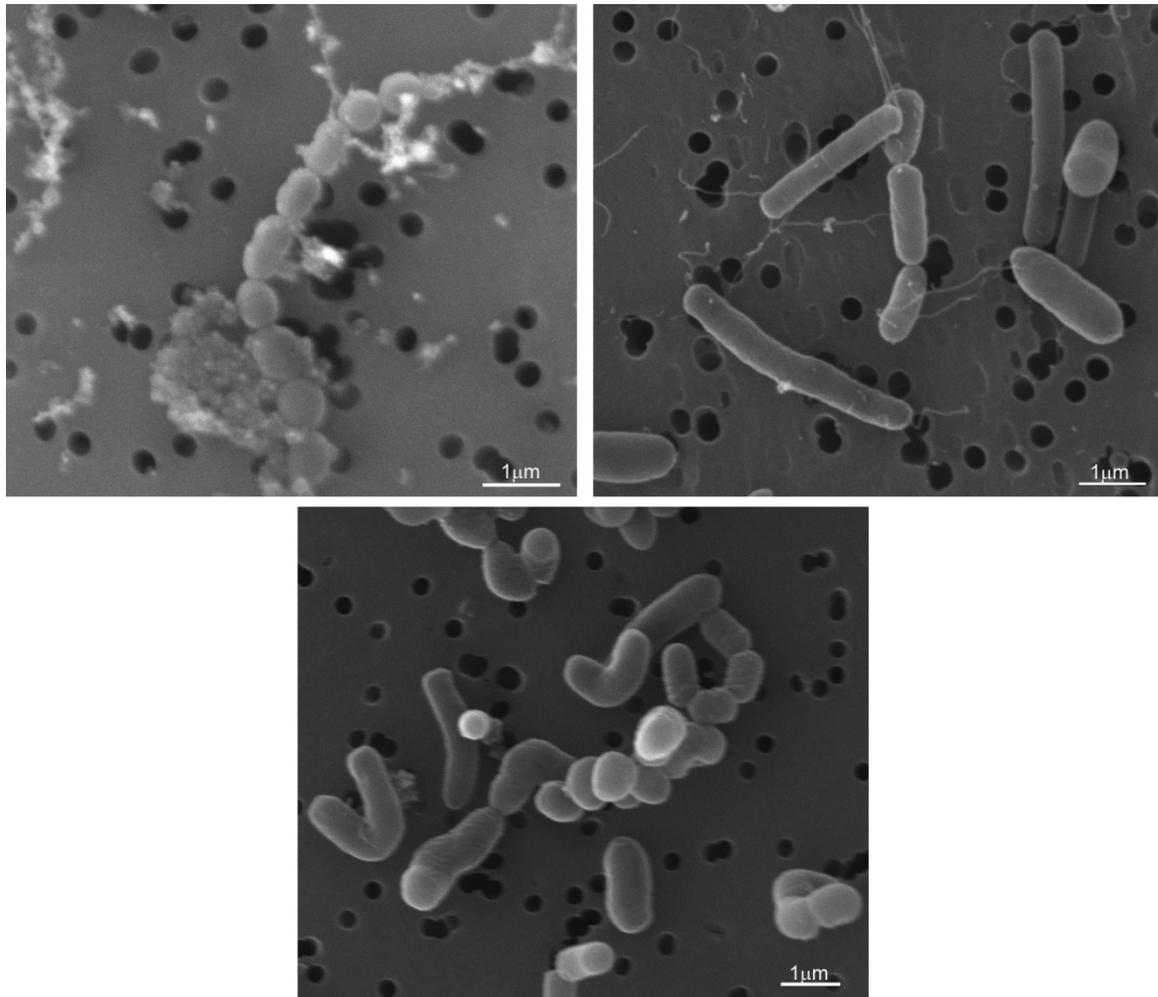
*S. mutans* and *L. rhamnosus* were grown in brain heart infusion broth and MRS broth, respectively, at 37°C under aerobic conditions. Aliquots of the two strains were combined, mixed by vortexing, and the spectrophotometric Kolenbrander coaggregation assay was run to establish baseline values for complete coaggregation [3,4]. An aliquot of a 0.08M solution of each sugar was added to samples of the combined bacteria and the coaggregation assay repeated. Absorbance values (650nm) from the assay were used to calculate the percentage of inhibition of coaggregation based on the protocol used by Riihinen, et al. in a study on the antiaggregation potential of berry fractions [5]. In this study, it was found that lactose produced 97% inhibition; D-galactose 90% inhibition; D-fructose 46% inhibition; and D-fucose 43% inhibition of aggregation as compared to control, untreated bacteria.

Coaggregation was also analyzed qualitatively by scanning electron microscopy. An equal volume of 5% glutaraldehyde in phosphate buffered saline (PBS) was added to samples of *S. mutans*, *L. rhamnosus*, and the coaggregated combination of the strains. The samples were passed through 0.2µm polycarbonate filters, rinsed with PBS and distilled water, dehydrated in a graded series of ethanol and critical point dried. Samples were mounted on aluminum stubs and coated with gold prior to imaging at 30 kV on a FEI Quanta 200 3D (Figure 1).

According to the calculated percentage values of inhibition of coaggregation, lactose showed the most inhibition of coaggregation of *S. mutans* and *L. rhamnosus*, while D-fucose had the lowest inhibition percentage. Commercial products containing these sugars should be evaluated for their ability to inhibit the production of dental caries.

## References

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**Figure 1:** SEM micrographs of *S. mutans* (top left), *L. rhamnosus* (top right), and coaggregating *S. mutans* and *L. rhamnosus* (bottom).