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**METHODS** 

# Study the action of enzyme alginate lyase on plaque forming bacteria (Streptococcus mutans)

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Students at Chandigarh University conducted an experiment to investigate the action of alginate lyase against plaque-forming *Streptococcus mutans*. In the experiment, students made two cultures. The first is a pure culture of *Streptococcus mutans*, and the second is a plaque biofilm containing all other microorganisms. Then another liquid culture of alphaproteobacteria is formed for the extraction of the alginate lyase enzyme. After the formation of bacterial cultures and the extraction of enzyme, a comparative study is done for the action of enzyme on culture A (*S. mutans*) and culture B (plaque biofilm). During the observations, students found that after the addition of alginate lyase to the system, *S. mutans* were unable to produce the polysaccharide matrix that made them the major bacterium responsible for the dental biofilm, and in culture B, a slight dispersion of biofilm was seen. Biofilm cannot adhere to solid surfaces due to the action of alginate ligase.

Keywords: alginate lyase, dental plaque, biofilm dispersion, enzymatic activity

## Introduction

Like other enzymes, lyase also functions as a catalyst for certain biochemical reactions like addition and elimination. Lyase breaks the bonds between atoms by elimination and by forming other bonds and rings. Alginate lyase (EC 4.2.2.3, EC 4.2.2.11) catalyzes alginate lyase<sup>1</sup> degradation via a beta-elimination mechanism. It breaks the glyosidic bond between monomers and produces a double bond between the carbons of the sugar ring. Alginate lyase is also known for its biofilm dispersion ability. Studies have shown that alginate lyase exhibits catalysis-independent biofilm dispersion.

Plaque biofilm: Our mouth is home to numerous microorganisms, and with poor oral hygiene, some bacteria, like. *Streptococcus mutans*, get stuck to the surface of teeth and produce large, strong colonies. These biofilms then arise as a dental condition called plaque. However, *S. mutans* plays a major role by helping in the production of the extracellular polysaccharide matrix.

## <sup>1</sup> It should be noted that alginate aids in the adhesion of bacteria to solid surfaces and also prevents bacteria from growing in unsanitary conditions.

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## **Experiment**

Aim: To study the action of alginate lyase on *S. mutans* and plaque biofilms.

Microbes required: Pure culture of *S. mutans*, mixed culture of biofilm, and liquid culture of rhizobiaceae.

## Steps involved

- (1) Isolation of pure *S. mutans* from the saliva of a person suffering from dental plaque and then preparation of a pure culture in sugar-rich culture media.
- (2) Plaque biofilm isolation from teeth with a toothpick and preparation of its mix culture in nutrient agar media.
- (3) Isolation of rhizobiaceae from root nodules and then growing it using potato extract broth before making its liquid culture.
- (4) Separation of alginate lyase from rhizobiaceae liquid culture by using the technique of centrifugation and the addition of urea solution.

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(5) Placing a pure culture of *S. mutans* (A) and a mixed culture of plaque (B) in an incubator with enzymes.

(6) Further, both plates were observed by the professors of the Chandigarh University, and comparative observations were made by comparing the blank plates and the cultural plates with enzyme.

## **Observations**

- (1) Plate A: The blank plate had a higher pH than the incubated plate, indicating that polysaccharide formation occurs in the blank plate, resulting in acid release. But due to the presence of alginate lyase in other plates, this reaction is hindered.
- (2) (*Streptococcus mutans* releases acid while making polysaccharides that cause cavities).
- (3) Plate B: Under a microscope, the mixed culture in the blank plate was forming a biofilm, whereas bacteria in other plates were scattered and unable

to form a biofilm due to the presence of alginate lyase.

## Results

Alginate lyase is effective against dental plaque.

## **Author contributions**

The author came up with the idea of performing an experiment on enzymatic activity. She chose the enzyme with anti-biofilm properties, as well as the study of dental plaque. Then she reached out to the professors of microbial diversity at Chandigarh University and got engaged in experimentation.

## References

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