

Identifying Genetic Mutations in *Streptococcus mutans* Required for Biofilm Formation and Tooth Attachment

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INTRODUCTION & PURPOSE

Streptococcus mutans contributes to dental caries by forming biofilms and producing enamel-damaging acids. The glycosyltransferase complex (*gtfBC*) is crucial for converting dietary sugars into glucan, allowing *S. mutans* to adhere to teeth and form biofilms. Our project builds on prior research that identified 90 *S. mutans* mutants with reduced biofilm formation. To determine if biofilm defects are due to mutations outside the *gtfBC* genes, we are using:

- DNA extraction
- PCR
- Gel electrophoresis

Mutants with intact *gtfBC* genes will be selected for genome sequencing. The goal is to identify new genes involved in biofilm production. This research could lead to novel strategies to prevent tooth decay and improve global oral health

DISCOVERY OF BIOFILM-DEFECTIVE MUTANTS

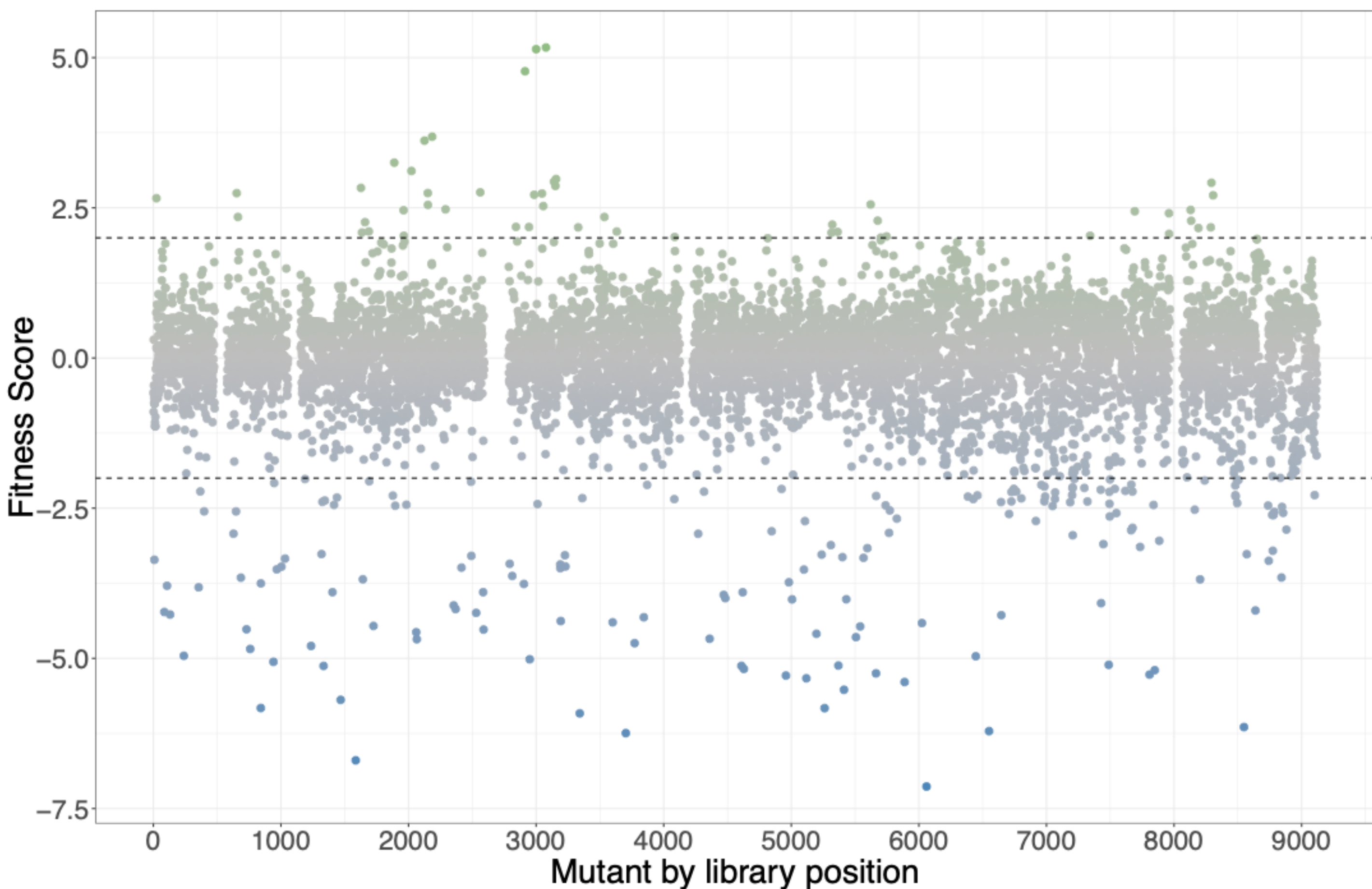


Figure 1.1 This study selected samples based on their Z-scores, calculated by Emanuel Cazano (B.S Biology), with a specific focus on biofilm formation. The lower the Z-score, the less biofilm that was present. Samples with Z-scores between -2 and -4 were chosen for further analysis. Samples with Z-scores below -4 were excluded, as they likely lacked the *gtfBC* genes. Previous studies have shown that the absence of these genes leads to reduced biofilm formation.

METHODS TO DETERMINE BIOFILM MUTATIONS

DNA Extraction

- Single colonies of *S. mutans* mutants were grown overnight in BHI broth.
- Cells were pelleted via centrifugation and resuspended in TE buffer.
- Lysozyme and proteinase K were added to break down cell walls and proteins.
- Using Biosearch Technologies DNA Purification Kit, DNA was isolated and purified.

PCR

- PCR was performed to amplify the *gtfBC* gene region.
- The PCR reaction mix included forward and reverse primers, LongAmp Master mix, nuclease-free water, and extracted DNA.
- Thermocycler conditions:
 - Initial denaturation (94°C, min)
 - 35 cycles of:
 - Denaturation (94°C, 10 sec)
 - Annealing (56°C, 30 sec)
 - Extension (65°C, 10 min)
 - Final extension (65°C, 10 min)
- This process amplified the *gtfBC* DNA segment

Gel Electrophoresis

- PCR products were loaded onto a 0.8% agarose gel
- A 1 Kb gene ruler was used to estimate fragment size.
- Electrophoresis was run at 120V for ~55 minutes in TAE buffer.
- Gels were visualized under UV light to confirm presence or lack of *gtfBC*.

Analysis:

Samples 52_H12, 34_D10, and 52_C4 have previously been associated with reduced biofilm production and contain intact *gtfBC* genes located at the 10 kbp region. Similarly, sample 2_C11 has also shown reduced biofilm production, however, it lacks the *gtfBC* gene entirely. In contrast, mutants 65_D12 and 71_B11 possess the *gtfBC* gene but have not been linked to any biofilm production. These two samples will serve as the primary focus of our study.

RESULTS FROM GENOME SEQUENCING

Strain	Z-score	Location of Tn Insertion	Unassigned missing coverage evidence	Previously linked to biofilm formation
2_C11	-4.27	SMU_545	<i>gtfBC</i>	Yes
34_D10	-1.87	SMU_833		Yes
52_C4	-2.18	<i>fruR</i>	SMU_436c	Yes
65_D12	-1.77	SMU_635	SMU_565c–SMU_566c	No
71_B11	-1.5	SMU_1280c	SMU_436c	No

Figure 2.1: The “Location of Transposon Insertions” column shows where insertions occurred in the genome.

- Insertions may disrupt gene function, even in non-coding regions, by affecting regulatory or nearby functional elements.

The “Unassigned Missing Coverage” column highlights genomic regions with missing genes, such as *gtfBC*.

Samples 52_H12, 34_D10, and 52_C4 were identified to possess the *gtfBC* gene on the gel electrophoresis and the DNA sequencing revealed a consistent link to Biofilm formation, which serves as evidence that the screen is functional.

52_C4 contains a transposon insertion in *fruR* (fructose repressor), a gene involved in biofilm regulation.

2_C11 is missing the *gtfBC* gene, though the exact cause is unknown.

Our research will further investigate mutants 65_D12 and 71_B11, which possess the *gtfBC* gene but have not been previously associated with biofilm formation. This will allow us to isolate and identify other genetic factors that may influence biofilm production.

FURTHER RESEARCH

- This project is part of a collaborative effort to understand biofilm formation in *S. mutans*.
- Graduate student Karen Solano is focusing on mutants not yet linked to biofilm defects, such as 65_D12 and 71_B11.
- She is characterizing these mutants by deleting or replacing genes with antibiotic-resistant cassettes.
- These cassettes allow for:
 - Easy selection on antibiotic-containing plates
 - Confirmation of successful gene editing
- Overall, this strategy brings us closer to identifying key genes responsible for reduced biofilm formation.

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