Causative Agents of Early Childhood Caries: Challenges and Solutions in Culturing and Sequencing

Daryn Erickson1,2, Ryann Whealy1,2, Jill Cocking1,2, Crystal Hepp1,2, Viacheslav Fofanov1,2
1Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, AZ
2Northern Arizona University, Flagstaff, AZ

Abstract

Dental caries, also known as cavities or dental decay, affects children at a rate five times higher than asthma in the United States. This disease is highly preventable but causes extreme pain and costs millions of dollars to treat every year. The presence of Streptococcus mutans and Streptococcus sobrinus plays a major role in the development and progression of tooth decay; therefore, it is important to establish colonization of these bacteria to assess the risk of developing the disease. Streptococcus bacteria are difficult to grow, extract, and sequence due to strict necessary growth conditions. In this study, we evaluated the performance of different storage and culturing protocols and developed strategies for reducing interference by unwanted bacterial and fungal genomes when sequencing extracted samples. We compared storage conditions of samples at various temperatures, and with and without glycerol. We decided the best storage method was at -80°C in a specialized solution known as Aimes. When sequencing cultures, we encountered various unwanted bacterial and fungal genomes. To reduce this, we modified our culturing methods by including growth in anaerobic conditions and using serial isolation streaking. These modifications have limited the growth of aerobic specimens and increased culture purity before extraction. With this study, we will be able to better understand the oral microbiome and aim to identify virulence factors in S. mutans and S. sobrinus that contribute to the high rates of dental caries in children.

Introduction

• Arizona leads the United States in rates of Early Childhood Caries (ECC)
• The presence of Streptococcus mutans (S. mutans) and Streptococcus sobrinus (S. sobrinus) bacteria plays a significant role in the development and progression of ECC
• Big Question: Why are Northern Arizona’s Children presenting with increasingly high rates of ECC?

Objectives

• Determine the best protocol for growing and isolating S. mutans and S. sobrinus to eliminate interfering DNA during sequencing
• Main Project Goal: Understand why Northern Arizona’s children have elevated rates of ECC

Study Background

Samples were collected from more than 40 preschools geographically distributed across Coconino County, AZ. Hygienists from the Smart Smiles Program travelled to the collection sites to clean the children’s teeth and were equipped with collection kits made by our lab to collect 180 plaque and saliva samples from children ages 1-5. Collection kits included 1 E-swab pre-packaged with the Aimes solution (as well as materials for plaque collection to be used in further studies). While the children were getting a dental cleaning, the saliva and plaque samples were collected then sent back to NAU for storage and analysis.

Storage and Culturing Testing

• Two different selective media were tested: SB-20 Media and TYCSB Media
• Storage at -20°C and -80°C was tested to determine if re-growth patterns differed between the temperatures
• Storage with and without glycerol for the saliva samples was tested to determine if the presence of glycerol in the storage solution made a difference in re-growing the bacteria

Isolation Testing

• Oral samples are plated on TYCSB Media and incubated in aerobic conditions for 48 hours
• Samples are extracted and determined to be positive/negative for S. mutans/S. sobrinus via a TaqMan® Assay
• S. mutans/S. sobrinus positive samples are plated on TYCSB Media and incubated for 48 hours
• Based off colony morphology, “strep-like” colonies are re-plated on TYCSB Media and incubated in anaerobic chamber for 48 hours
• “Strep-like” colonies present after re-isolation are extracted and sequenced

Results

• We found no growth differences when comparing the storage conditions and the growth medium. Due to this, we chose to store the E-swabs at -80°C with no glycerol added to the storage solution. We also chose to use TYCSB Media to grow the bacteria
• Anaerobic conditions sometimes inhibited the growth of interfering bacteria. However, using the anaerobic chamber has not led to an increased ability to isolate S. mutans or S. sobrinus. This has led to interfering genomes still being present during sequencing
• Performing multiple streaks increased culture purity sometimes. However, isolating S. mutans and S. sobrinus hasn’t been made easier with serial streaking

Conclusions

• All tested storage, and growth conditions were successful in producing bacterial growth. Therefore, the storage, and growth conditions chosen were based on convenience and efficiency
• We are successfully growing both S. mutans and S. sobrinus. However, we are still seeing complex cocci environments and still facing issues with isolating these bacteria
• We are still sequenc[ing] additional bacteria along with our targets which is expected since isolating S. mutans and S. sobrinus has proved very difficult
• Despite growth and isolation problems, carriage rates are consistent with our expectations. We have a 49% carriage rate currently after processing 180 samples

Acknowledgements

Coconino County Public Health Services District
Katy Baszar, Smart Smiles Program
Heather Williams, Smart Smiles Program
Robin Ieven, RDH, Smart Smiles Program
Tony Zarate Garcia, RDH, Smart Smiles Program

Daryn Erickson, Ryann Whealy, Jill Cocking, Crystal Hepp, Viacheslav Fofanov
Northern Arizona University
Flagstaff, AZ

This work has been supported by the Arizona Biomedical Commission New Investigator Award #N001624-15-3277. Preliminary results for this project were supported by Arizona Technology Research Initiative Fund, Research Development Grants Program