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Original article

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Streptococcus Mutans Serotype Analysis from Dental Plaque of Caries Patients in Banda Aceh Based on the GTF Gene

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Abstract

Background: Dental caries is an oral disease that is widely suffered by the population of Aceh caused by *Streptococcus mutans*. *S. mutans* serotypes c and d are widely isolated in the human oral cavity. This research was focused on detecting the presence and variability of *S. mutans* in supragingival dental plaque of caries teenager and young adults' patients.

Methods: Subjects involved in this study were patients who treated at the Rumah Sakit Gigi dan Mulut of Dentistry Faculty of Universitas Syiah Kuala. The approach used in this research was molecular microbiology technique. To determine the presence of S. mutans, supragingival plaque from caries patients was cultivated in TYS20B. The culture findings were utilized to detect the presence of bacteria using PCR. The primers utilized in the PCR were S. mutans specific primers, GTFB (517 bp) for S. mutans serotype c and GTFI (712 bp) for S. mutans serotype d.

Results: Culture results on TYS20B media showed the growth of *S. mutans* colonies isolated from the supragingival plaque of research subjects. PCR results also revealed the presence of S. mutans in the supragingival plaques of caries patients, with the variability of S. mutans discovered to be a serotype c and a serotype d.

Conclusions: Based on the findings of this study, it can be concluded that S. mutans can be found in the supragingival plaques of caries patients with the serotypes c and d variability.

Keywords: Cariogenic, Dental Caries, *Streptococcus mutans*, Supragingival Plaque, TYS20B.

Introduction

Aceh Province is one of the provinces in *Indonesia* with a high caries prevalence rate. Caries can arise at various ages, social levels, and levels of education (1-3). According to the American Academy of Pediatrics, oral infections continue to afflict children, particularly very young children. Efforts to combat the disease must be coordinated and include preventive, curative, rehabilitative, and promotional measures. To provide an effective result, the medications used must be compatible with the microorganisms that cause caries. Previous studies found a link between the presence of *streptococcal mutans* in tooth plaque. As a result, the density of mutans streptococci in dental plaque predicts caries risk (4,5).

The main causative agent causing dental caries is *Streptococcus mutans* (*S. mutans*). *S. mutans* is the most isolated bacterium species from the human mouth and adheres to the tooth (6). This species is part of the oral flora; however, it has a role in the demineralization of teeth, causing tooth disease known as dental caries. Dental caries is an infectious illness that damages the teeth's hard tissues, notably the

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enamel and dentin, and is produced by a combination of factors such as substrate, host, time, and bacteria (7). These bacteria are also capable of causing endocarditis (8,9).

Bacteria adhering to the tooth surface will colonize and accumulate with the assistance of *glucosyltransferase* (Gtf) and *glucan binding protein* (Gbp) (10,11). The Gtf enzyme catalyzes the synthesis of *water-soluble glucan* (glucan of (1-6) linked glucose groups) and *water-insoluble glucan* (glucan of (1-3) bonded glucose groups) from sucrose breakdown, and Gbp is a glucan-binding receptor protein (12,13).

Streptococcus mutans and other normal flora species can be isolated from saliva, mucosal surfaces, and plaque that collects in tooth cavities or on the surface of healthy teeth (14,15). *S. mutans* can induce pathological alterations in the mouth (caries) as well as systemic infections (coronary heart infection/endocarditis). A person's immune status, on the other hand, is also determined by age, and in this case, the age of a toddler and/or the elderly can be classified as susceptible to infection (16,17).

Although the role of *S. mutans* in the process of tooth enamel demineralization is clearly known, this species can also be isolated from the subject's mouth with good oral hygiene without caries (18). In other words, the variety of strains in a person's mouth influences microbe coaggregation with other flora in the oral ecosystem. Thus, there is a possibility of clonal types that dominate certain habitats (oral niche) in the mouth in the case of S. mutans as part of oral flora on the one hand and as the cause of caries on the other (19,20).

The objective of this research was to detect the presence of *S. mutans* in the mouths of susceptible subjects and to examine its variability based on age differences in patients with dental caries. It was hoped that the findings of this study will aid in the development of preventive measures (such as vaccinations or competitive exclusion procedures) against germs that could cause this systemic disease.

Materials and Methods

Subjects

The stages carried out in this study were started with the determination of the research subject. Subjects involved in this study were subjects who treated at Rumah Sakit Gigi Mulut of Dentistry Faculty, Universitas Syiah Kuala, did not experience systemic disease and were not currently receiving antibiotic therapy. Selection was made after obtaining ethical approval. The research was started with the selection of research subjects and requests for approval/willingness of the subject or parents to participate in this research activity. Willingness to participate in the subject was stated in writing by signing the informed concerned sheet provided.

Sample collections

The next step was to take supragingival plaque and detect the presence of *S. mutans* in it. The sample collection was done by using sterile sonde. The sample was put into a tube containing the transport media to be subsequently taken to the Dental Laboratory Skill of Dentistry Faculty, Universitas Syiah Kuala to detect the presence of *S. mutans* in supragingival plaque. The presence of *S. mutans* in the subject's supragingival plaque was done in 2 ways namely bacterial culture on selective media namely Tr*ypticase Soy with Sucrose and Bacitracin* (TYS20B) and PCR by using specific primers that tracked glucosyltransferases (Gtf).

Detection of S. mutans by Polymerase Chain Reaction (PCR)

The *Polymerase* Chain *Reaction* (PCR) method was used to identify the variability of *S. mutans* serotypes. *S. mutans* serotypes were identified using specific primers capable of detecting the presence of each of the serotypes listed in Table 1. The programme is shown in Table 2.

The last stage of this study was the detection of *S. mutans* variability from the supragingival plaque of the subjects by the PCR method. This variability examination was first carried out by culturing the *S. mutans* that grew on

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TYS20B in liquid media, namely *Trypticase Soy Broth* (TSB). *S. mutans* that grow on liquid media was used to isolate *S. mutans* DNA. The isolation process was carried out by extracting *S. mutans* DNA which was carried out according to the procedure recommended by the TIANamp Bacteria DNA Kit (Tiangen) and in the extraction process lysozyme was used because *S. mutans* was a Gram-positive bacterium.

Table 1. Primary sequence for *Streptococcus mutans* serotyping.

Primer	Sequence	bp	Serotype
GTFB-F	5'-ACTACACTTTCGGGTGGCTTG G-3'	51	c
GTFB-R	5'-CAGTATAAGCGCCAGTTTCATC-3'		
GTFI-F	5'-GATAACTACCTGACAGCTGACT-3'	712	d
GTFI-R	5'-AAGCTGCCTTAAGGTAATCACT-3'		

Table 2. Amplification condition of *Streptococcus mutans* DNA using PCR.

Step	Temperature (°C)	Time	Cycle
Initial Denaturation	95	3 minutes	1
Denaturation	95	1 minute	3
Annealing	57	30 second	
Elongation	72	30 second	
Final Elongation	72	5 minutes	1

The extracted DNA was used as a template to be mixed with PCR Master Mix. The PCR tube was filled with a master mix PCR (12.5 µl), forward and reverse specific primers (2.5 µl each) and DNA template (3.5 µl) and H2O to reach a volume of 25 µl per tube. The filled PCR tube was then placed in a PCR machine namely thermal cycler (BioRad Lab, USA) for DNA amplification. Amplified DNA samples were analyzed by electrophoresis using 1.2% agarose. To run electrophoresis, a sample of 7 µl running PCR was taken which was injected into wells in 1.2% Agarose, one well was injected as a guide marker. Electrophoresis was run with a current of 100 mA and a voltage of 100 volts for 30 minutes. To see the PCR product produced, the Agarose was photographed using a gel documentation system for visualizing DNA bands. The results were then analyzed to see the variability of *S. mutans* from caries patients with different age groups and the severity of caries.

Results

Subjects and samples

The selection of research subjects yielded 34 patients who met the criteria for supragingival plaque retrieval. Bacterial culture of caries patients with supragingival plaque on selective media for the *S. mutans* species, TYS20B, was performed.

PCR

The results of DNA amplification showed the presence of DNA bands at 517 bp and 712 bp (Fig. 1).

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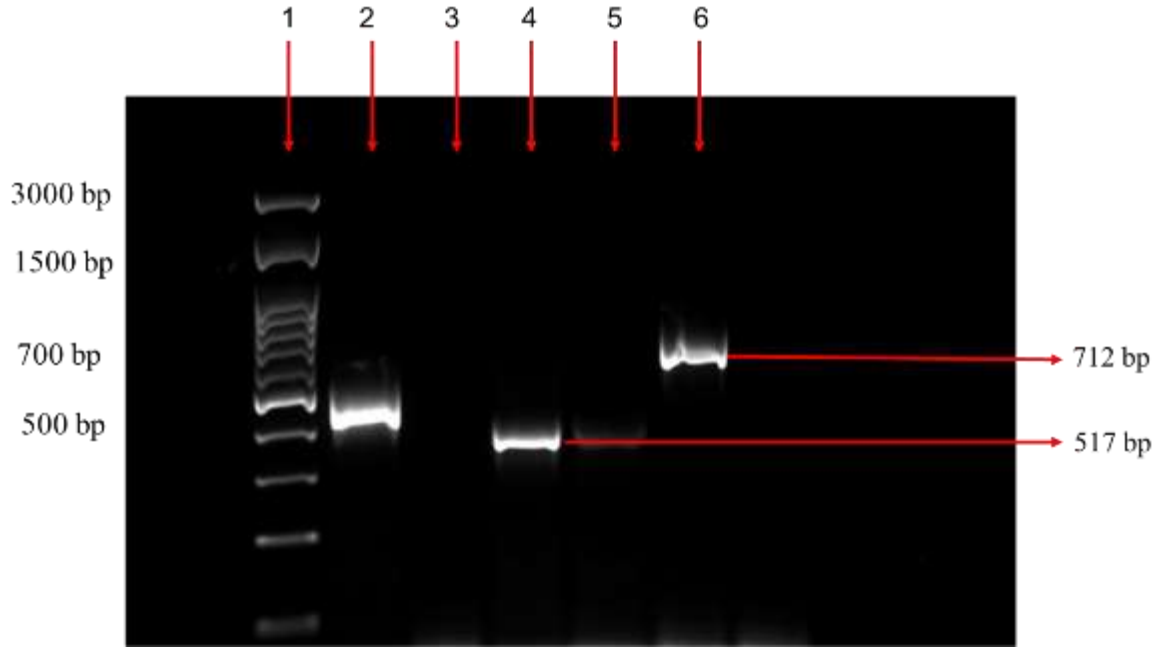

Fig. 1. *S. mutans* DNA bands amplification results by PCR. The lane 1 is a DNA marker, lane 2 is a positive control, lanes 3,5,6,8,9 are *S. mutans* serotype c DNA bands, and lanes 4,7,10 are *S. mutans* serotype d DNA bands.

Discussion

Streptococcus mutans is a Gram-positive bacterium that grows in a facultative anaerobic atmosphere that requires 5%-10% CO2 and 95% nitrogen and ammonia to survive (21,22). *S. mutans* will only become pathogenic under conditions of frequent and prolonged acidification of dental plaque. *S. mutans* is adapted to low pH and will increase the rate of acid production and drive the pH even lower, resulting in cariogenic plaque (23).

Trypticase Soy With Sucrose and Bacitracin (TYS20B) is one of the selective media for *S. mutans* (21,24). The culture results on TYS20B in this study demonstrated *S. mutans* colony characteristics, such as round colonies with flat edges, yellowish white colour, and a distinct yeast-like aroma. TYS20B selective media can separate streptococci mutant group bacteria, in this case *S. mutans*, from other bacteria in oral plaque. There were no morphological differences in colony growth between *S. mutans* serotypes c and d colonies. *S. mutans* serotype c is the most common serotype isolated from caries patients' plaque and saliva (25,26).

This serotype classification is based on the percentage of guanine and cytosine in the DNA. The extracted DNA was used as a template for PCR, and the PCR products were visualized using electrophoresis. The visualization results revealed the presence of *S. mutans* in the study subject's plaque, which was indicated by the presence of bands or DNA bands based on markers with weights of 712 bp and 517 bp (Fig. 1). The figure shows that lane 1 is a DNA marker, lane 2 is a positive control, lanes 3,5,6,8,9 are *S. mutans* serotype c DNA bands, and lanes 4,7,10 are *S. mutans* serotype d DNA bands. The DNA amplification results obtained for all supragingival plaque samples (34 subjects consisting of 16 subject’s teenager and 19 subjects’ young adult).

The use of *Trypticase Soy with Sucrose and Bacitracin* (TYS20B) selective media to separate *S. mutans* species from other bacteria present in supragingival plaque was very effective and efficient. The presence of sucrose at a very high concentration of 20% in TYS20B was responsible for its ability. Only *S. mutans* species grew on TYS20B media at high sucrose concentrations. Furthermore, after 3 x 24 hours of incubation in anaerobic conditions, the *S. mutans* colony could grow

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on the selective media. This significantly reduced the time required to grow and identify *S. mutans*. *S. mutans* in the human oral cavity is known to have two serotypes, c and d.

In this study, specific primers were used to trace *glucosyltransferase* (GTF) to detect the presence of *S. mutans* serotypes c and d. Gtf, encoded by the gtf B gene, is a major virulence factor of *S. mutans* that functions to synthesize glucan from sucrose (27). Glucan is required for bacterial attachment to the tooth surface, which leads to the formation of a dental biofilm known as dental plaque. In this study, the serotyping results of *S. mutans* revealed that serotype c was the most common serotype, followed by serotype d. This is consistent with the theory that serotype c is the most common. This is consistent with the theory that *S. mutans* serotype c is the most commonly isolated serotype from caries patients when compared to other serotypes (25,26).

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Conflict of Interest
The authors declare no potential conflict of interest regarding the publication of this work. In addition, the ethical issues including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely witnessed by the authors.

Ethics Code
The authors approved this study in Ethical Number: Ref: 75/KEPH/XII/2020.

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