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Molecular Detection Of Bifidobacterium Dentium From Patients With Tooth Caries

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Abstract:

The goal of this study for molecular detection of Bifidobacterium dentium from patients with tooth caries.Samples were taken from the root caries lesions of nine distinct patients, which yielded 150 samples. fastidious anaerobe broth was used to dilute the samples.Then identifications of bacteria were done by using biochemical tests. DNA of suspected bacteria were extracted and two primers were used for detection of B. dentium (CI and 16S rDNA). According to conventional techniques and biochemical analyses, 28 isolates were B. dentium. whereas molecular identification identified 24 (85.7 percent) of the isolates as being of B. dentium. To find particular genes, all the isolates in this investigation were subjected to PCR using species-specific primers and a PCR method called conventional PCR.

In conclusion, B. dentium was detected in a high percentage in dental caries patients which were diagnosed by .bacteriological and molecular methods.

Key words: B. dentium, Tooth caries, PCR.

Introduction:

Dieto-bacterial dental caries is the most frequent oral cavity illness in children and a major public health issue across the world. Cariogenic flora and cariogenic foods combine to cause the disease. Koch's postulates led the majority of previous researchers to concentrate their efforts on a small number of microorganisms in pure cultures. Even yet, the exact origin of a severe case of dental caries is a mystery. acidic and acid-resistant microorganisms have been found in the majority of studies (Xu et al., 2014).

Bacteria from both animals and people have been used to create the Bifidobacteriaceae family of Bifidobacteriums, which includes the genera Aeriscardo, Alloscardo, Bifidobacterium, Gardnerella et al. Gram-positive anaerobic bacilli are found mostly in the digestive system of humans (Scardovi, 1986). In addition to lowering the quantity of dangerous bacteria in the host, they have been shown to increase gut immunity (Gill et al., 2001).

Bifidobacterium has been identified in the mouth in recent studies (Beighton et al., 2008). Bacteria from the oral biofilm Bifidobacterium dentium and longum, two of the ten species, have been detected in saliva (Modesto et al., 2006). "(Beighton and colleagues, 2008); A research detected Bifidobacterium in 80% of plaque samples from young children with caries (Aas et al., 2008; Tanner et al., 2011).Children with caries had a greater prevalence of Bifidobacterium in their saliva than those without the disease (95% vs. 9%). (Kaur et al., 2013). A new caries-causing bacteria has been identified as oral Bifidobacterium based on these findings. B. dentium is the most prevalent kind of Bifidobacterium in the oral cavity (Munson et al., 2004). Additionally, B. longum may be found in the human oral cavity on a regular basis (Nyvad and Kilian,1990; Aas et al.,2008; Mantzourani et al.,2009).

It has been shown that these bacteria are very acid-resistant as a consequence They have a superior survival rate than Streptococcus mutans and Lactobacilli paracasei when it comes to intracellular pH. (Nakajo et al., 2010). It is one of the most important caries-inducing factors because bacteria's ability to create acids under acidic settings is one that is acquired from the breakdown of carbohydrates.According to recent research, the so-called "bifid shunt" is a unique metabolic route found only in Bifidobacterium species. Glycolysis (Embden-Meyerhof-Parnas pathway) is the most common method of metabolizing carbohydrates in bacteria that cause dental caries, such as S. mutans. Acetate and lactate are both produced by Bifidobacterium species when they metabolize carbohydrates through the "bifid shunt." There is still a lot to learn about how caries-related B. longum and B. dentium digest carbohydrates and produce acids in the mouth.The goal of this study for molecular detection of Bifidobacterium dentium from patients with tooth caries.

Materials and patients:

Samples were taken from the root caries lesions of nine distinct patients, which yielded 150 samples. fastidious anaerobe broth was used to dilute the samples. It was necessary to use modified MTPY medium in order to isolate bifids from the aliquots of samples. The medium

was autoclaved at anaerobic conditions. Then identifications of bacteria were done according to the (Markey et al., 2013)

DNA of bacteria were extracted by using Promega kit, the method of extraction was done according to the manufacturer instructions.

Primers:

Two primers were used for detection of B. dentium:

CI F: 5'-GTS CAY GAR GGY CTS AAG AAG CAG GAA AGA ACA TGT GAG CA-3'

CI R: 5'-CCR TCC TGG CCR ACC TTG TAC GAC CTA CAC CGA ACT GAG A-3'.

16S rDNA

F (5'-GAAGAGTTTGATCCTGGCTCAG-3') 700 bp

R (5'-CTACGGCTACCTTGTTACGA-3').

Two milliliters of template DNA, two milliliters of particular primers, ten milliliters of 2x Taq master mix, and eleven milliliters of PCR grade water were used to make the PCR mixture used in the experiment. When it came to doing PCR, a heat cycler was used.

An all-purpose PCR method was used to CI gene up the DNA samples. Initial denaturation took 5 minutes at 94°C, followed by 1 minute at 54°C annealing, 1 minute at 72°C extension, followed by a final 7minute extension at 72°C. This was followed by 35 cycles of denaturation, extension, and annealing at 72°C.

For PCR method of 16S rDNA, only different in the annealing temperature were 56 °C.

Electrophoresis agarose gel was used to examine DNA samples, ladder of 100bp was used.

Results:

According to conventional techniques and biochemical analyses, 28isolateswereB. dentium. whereas molecular identification identified 24 (85.7 percent) of the isolates as being of B. dentium(Table 1). To find particular genes, all the isolates in this investigation were subjected to PCR using species-specific primers and a PCR method called conventional PCR (Fig. 1, 2).

Table 1. Numbers of bacteria isolated by traditional methods VS molecular methods

Method	No. B. dentium	percentage

Biochemical method	**28**	**18.7**
Molecular method	**24**	**85.7**

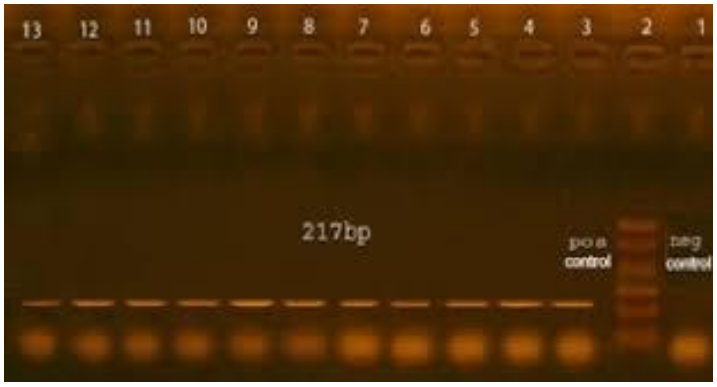

Figure 1. PCR of B. dentium at 217 bp of CI gene. Lane M represents 100 bp DNA marker.

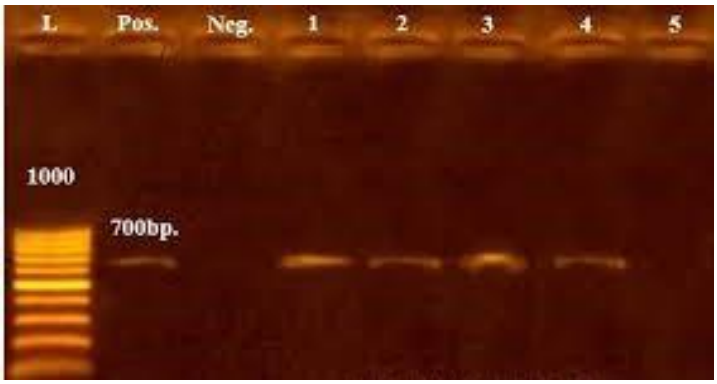

Figure 1. PCR of B. dentium at 700 bp of 16S rDNA. Lane M represents 100 bp DNA marker.

Discussion:

It has been observed that bifidobacteria that are both acidogenic and acidiuric are oral cavity commensal microorganisms, even if their importance in oral health is still up in the air. They've been linked to the development of cavities in children, adolescents, and adults, as well as the elderly (Beighton et al., 2010; Mantzourani et al.,2009). When the microbiology of occlusal caries lesions was investigated, bifidobacteria were detected exclusively at the areas of active decay (Dige et al., 2014).

Bifidobacteria may be isolated from saliva, plaque, and caries lesions samples (Mantzourani et al., 2009).

In our investigation, Bifidobacterium dentium (B. dentium) was detected in 18.7 percent of dental caries patients, which is less than our prevalence of 30.8 percent (Henne et al., 2015).

For example, PCR and oligonucleotide probes have been used to detect Bifidobacterium strains (Kullen et al., 1997, Matsuki et al., 1999, Requena et al., 2002). PCR-based techniques have been used to identify a single or a few species in most research. Group-specific polymerase chain reaction (PCR) approaches have recently been developed to identify Bifidobacterium species from a wide range of sources (Matsuki et al., 1998; Mullie et al., 2003). Until now, no PCR approach has been able to differentiate more than five species at a time. Individual primer sets based on 16S, 23S rRNA gene or ISR sequences were blamed for this (Leblong-Bourget et al., 1996). In order to identify a wide range of potentially probiotic Bifidobacterium species using a single PCR test, our research focused on developing multiplex primer sets for each of these species.

When DNA-DNA hybridization, 16S rDNA sequence analysis and particular probe hybridization were initially established as molecular identification procedures, they were called DNA-DNA hybridization, 16S rDNA sequence analysis, and hybridization with specific probes. Molecular identification is distinct from physiological and biochemical features in that nucleic acid composition is used as a basis for identification.

It is common to utilize molecular approaches in conjunction with traditional microbiological identification. It is possible to measure DNA-DNA hybridization by utilizing membrane filters for DNA fixation and radioisotopes for detection (Crociani et al., 1996), or by using a microplate and photobiotin (Yaeshima et al., 1996).

Conclusion:

B. dentium was detected in a high percentage in dental caries patients which were diagnosed by bacteriological and molecular methods.

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