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RESEARCH ARTICLE

Isolation and Molecular Characterization of Lactobacillus Genus from Patients Wear in Fixed Orthodontic Appliance

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Abstract

Background: The fixed appliances might prevent effective oral hygiene and cause high cariogenic challenge. Objective: This study was examining the presence of lactobacillus *Spp* by traditional culture and PCR methods. Material and Method: A total 74 patients with age range from 15 to 35with mean age (19.67) that enrolled in this study. The samples were collected from patients admitted to orthodontic department in collage of dentistry during the period from November 2017 to March 2018. The result: The result were shown 59 of 74 was positive result, Thirty eight of 59was positive in PCR technique while 21of 59 positive in culture ,The methods of PCR and growth detected this bacteria was only 35.6%positive in culture ,while PCR methods were 64.4%%. Conclusion: the higher level of bacteria detected by PCR than routine culture method confirm that the PCR method is very sensitive, specific and time consume technique for detecting of oral bacteria.

Keywords: *Orthodontic Appliances, Lactobacillus, Polymerase Chain Reaction, Biofilm formation, Culture.*

Introduction

Oral dental plaques are biofilms composed of numerous genetically distinct types of bacteria that live in close collocation on oral surfaces. The most common bacteria associated with dental plaque are Streptococcus and Lactobacillus [1]. Scientific publications have demonstrated that the presence of fixed appliances in the oral cavity of orthodontic patients could alter the nature of dental plaque. The structure, metabolism and composition of dental plaque would change, leading to an increase in microbial population, especially Lactobacillus Spp. These conditions favoring microbial colonization and establishment of Lactobacillus spp. that increase microbial population growth and plaque accumulation [2, 3].

This genus is involved in the progression of the carious lesions and carious dentin is the main ecological site of lactobacilli [4]. Members of the Lactobacillus genus are characterized as Gram-positive, facultative anaerobic, non-motile, nonspore-forming, rod-shaped and catalase-negative. Techniques for identifying the species include: carbohydrate fermentation, arginine hydrolysis and enzyme activity. Since such biochemical methods depend on environmental and culture conditions, they sometimes lead to ambiguous results or even misidentifications [5].

Material and Method

Samples were collected from patients admitted to orthodontic department in collage of dentistry in hila city; the samples were composed from both sexes, during the period from November 2017 to March 2018.age group (15-35)years with mean age(19.67). Swabs were taken from patients with dental plaque cases were selected for inclusion in the study. Patients who do not put the orthodontic device and take medication or suffering from systemic diseases are excluded from the study. The samples used to this study were collected from the tooth of the patients diagnosed with dental plaque.

Detection of Bacterial by Culturing

For each sample, swab was inoculated on selective medium deMan Rogosa Sharpe

(MRS) agar [6].Was incubated an aerobically at 37 C° for 48 hrs, that promotes the growth of Lactobacilli while suppressing growth of other bacteria. Presence of lactobacillus was determined by culturing and biochemical test.

Detection of Bacteria by PCR

Identification of Lactobacillus to the genus level was done using PCR assay.

When a DNA from the Lactobacillus isolates was used as a template, a 250 bp [7]. DNA extraction was performed according to protocols recommended by manufacturer (Promega/USA), after bacterial DNA extraction from the clinical samples, PCR was performed to detect positive samples using specific primers for the 16S ribosomal RNA gene. By using electrophoresis with Agarose gel (1.5%) stained by ethidium bromide (0.5 mg/ml), amplified products were analyzed.

Table1: Specific Primers lactobacillus gens sequence and amplicon size

	Primer sequence (5′-3′)	Amplicon size(bp)	Reference
Gene Primer of Lactobacillus Genus	5'-CTCAAA ACT AAA CAA AGT TTC-3' 5'-CTT GTA CAC ACC GCC CGTCA-3'	250	(Gad et al., 2014).

Detection of Virulence Factors of Lactobacilli

Adherence Ability Test

The ability of lactobacilli to adhere to oral epithelial cell is one of important virulence properties of these bacteria and detected according to [8].

Biofilm Production

Tissue culture plate method (TCP) assay (also called semi quantitative micro titer plate test (biofilm assay) described by [9] was most widely used and was considered as standard test for detection of biofilm formation.

Results

A total of 74 patients wearing orthodontic appliance were enrolled in the present study. Dental plaques samples taken from these patients with age range (15-35) years. Also founded that 59 of 74 sample was positive result. Founded that 21 and 38 of 59 was positive results in culture and PCR technique respectively. Associated with orthodontic appliances. In the present study, 16S rRNA gene sequence analysis showed higher sensitivity to detect specific bacteria (64.4%) than the usual culture method (35.6%).

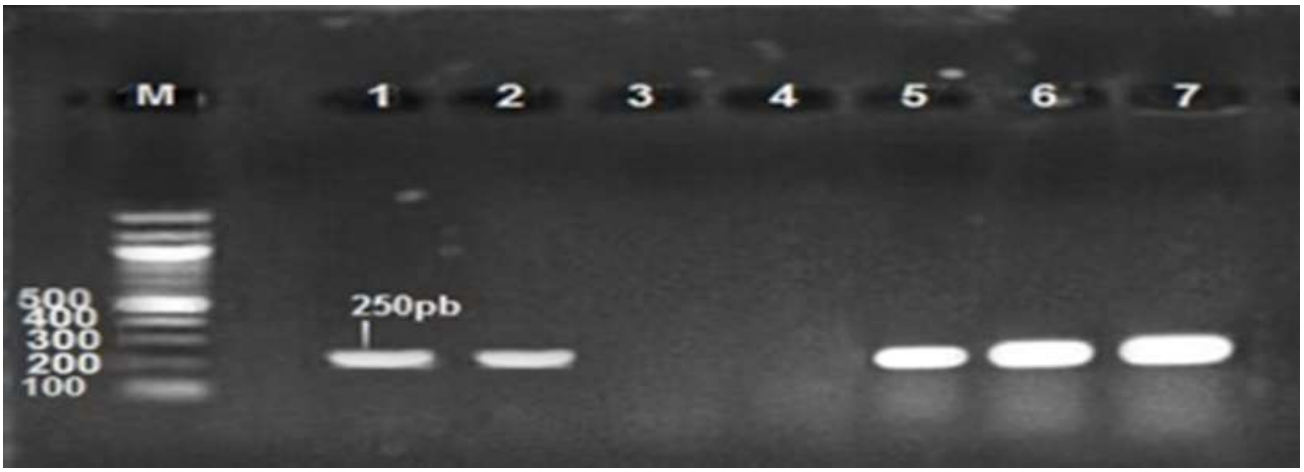

Figure 1: 1.5% Agarose gel electrophoresis at 72 volt for 60 minutes of PCR to lactobacillus general amplicon (250bp); lane M represents DNA marker size (100bp). Lane (1-7 except 3&4) represent positive isolates

Virulence Factors of Lactobacillus

Production of Biofilm in Lactobacillus *Spp*

As shown in Table (3-4)the results revealed all lactobacillus isolates were biofilm former, high and moderate biofilm formation mode were account for (100%) while there is no isolates that express non biofilm formation.

Table 2: Production of biofilm in lactobacillus

Bacterial isolate No.	Biofilm			
	Strong	Moderate	weak	% of biofilm formation
Lactobacillus (10)	4(40%)	4(40%)	2(20%)	100 %

Adherence Ability

Figure (1) show that Adherence of bacteria to the oral epithelial cells is the first step in the pathogenesis of bacterial infection and facilitated by action of several adhesions located on the surface of bacteria. The results showed that all lactobacillus *Spp* have ability to adherence to oral epithelial cells.

Discussion

These results harmonize to result by [10, 11] who found that bacterial identification was dependent on culture-based techniques. However, cultivation does not provide a full picture of the complex and diverse plaque bacterial community because a large number of oral bacteria are not amenable to culture-methods available. Despite the limitation of this approach, nearly 500 bacterial strains have been recovered from the sub gingival crevice [12]. Although PCR this is the most commonly used technique in molecular-based oral bacterial due to its simplicity, sensitivity, quick, accurate and cost-effectiveness .that harmonize with results by [13].

Which register that 16s rRNA gene sequencing enables the identification of rare bacteria with unusual phenotypic profiles, slow growing bacteria, novel bacteria, diagnosis of culture-negative infections alongside routine bacterial identification. Also found that PCR method more sensitive than culture method. Members of the genus Lactobacillus are commonly present as members of these communities and have received considerable attention with respect to their putative health-conferring properties as probiotics [14] Biofilm facilitates the adherence of microorganisms to biomedical surfaces and protect them from host immune response and antimicrobial therapy [15].Complicated appliance designs with loops and auxiliary arch wires create areas that are difficult to clean and may therefore enhance biofilm formation Bacterial biofilm infections are particularly problematic because sessile bacteria can withstand host immune responses and are drastically more resistant to antibiotics (up to 1000-fold), biocides and hydrodynamic shear forces than their planktonic counterparts [16].

Polysaccharides such as glucanic and dextranic acid polymer had been identified as part of the extracellular matrix, which plays an important positive role in building the mature biofilm structure [17]. Also [18] they were showed the production of exopolysaccharides is a key factor in the adherence of dental biofilm. Among some lactobacilli species, it has been studied extensively in the food industry, however there are fewer studies concerning oral lactobacilli. As adherence is one major factor in the formation of dental plaque, some authors have investigated in vitro the correlation between the presence of lactobacilli in dental plaque and their capacity to coagregate with other species.

Conclusion

The Lactobacillus was successfully isolated after series of purification. It was identified as lactobacillus genus and PCR assay provides a more rapid, specific and sensitive alternative to conventional culture methods for the identification of Lactobacillus.

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