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Correlation between DMFT index andoccurrence of *Lactobacillus* spp. in the oral cavity

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ABSTRACT

The human oral cavity includes different bacterial community. Advantage and disadvantage of these bacteria depend on their ability to adhere to the tooth surfaces. Some microbiota could cause oral disease, however, some of them inhibit formation of biofilm on teeth and therefore prevent dental caries. The present study was undertaken to investigate on the DMFT index (D: decay, M: missing, F: filling, T: teeth) and the occurrence of different species of *Lactobacillus*, as the most important oral microbiota. Ninety swab samples were collected from saliva and teeth of the patients and subjected to microbiological analysis. Along with phenotypic and genotypic identifications of the isolates, the DMFT index of each patient was measured to determine the oral conditions. To continue the study the antibacterial effect of five commercial toothpastes was evaluated on the isolates. The results obtained indicated that the frequency of occurrence of *L.plantarum*, *L.rhamnosus* and *L.mali* was high in the patients with a low DMFT score and *L.otakiensis*, *L.diolivorans* and *L.kefiri* was high in the patients with a high DMFT score. Althoughall isolates were susceptible to the commercial tooth pastes, *L.plantarum* and *L.rhamnosus* were relatively more sensitive. Based on the results, human oral health might be affected by the population of Lactobacillusspp. Furthermore, use of toothpastes without adequate information concerning to their antimicrobial effects might eliminate the population of beneficial oral microbiotaand hence increase the risk of oral diseases.

1. Introduction

Nowadays, human health might be dependent on the presence of lactic acid bacteria in food. This phenomena for the treatment of gastroenteritis was recognized in before century (BC) (Bottazzi, 1983). The term Probiotics refers to animate microorganisms which, when administrated in adequate numbers, may have beneficial effects for human health (Jindal et al., 2012). Bacteria mold and yeast may be used as probiotics. The most famous probiotics are Lactobacilli, Bifidobacteria and *Saccharomyces cervisiae*. Probiotics may have beneficial effects in several parts of the human body, including the digestive system, which could be considered a highly affected system. The oral cavity is the first part of the digestive system, which includes large microbial community (Faveri et al., 2006). It is estimated that more than one thousand bacterial species exist in the mouth (0.1% are present on the tongue) (Keijser et al., 2008). Nowadays, oral cavity health might be related to the number and type of organisms that compose themicrobiota. Conversely, it is clear that the formation of biofilm on the surface of teeth

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causes decay. In this regard, bacterial ability for adhesion to the surface of teeth considered an important virulence factor (Mohanty et al., 2012). Therefore, prevention of bacterial adhesion is the first stepin minimizing tooth decay (Burne et al., 1999). In general, saliva and bacteriocins eliminated the adhesion. Saliva is excreted by the host, and bacteriocins are produced by some probiotics viz., *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus rhamnosus* and *Lactobacillus salivarius* (Haukioja et al., 2006). The DMFT index (D: decay, M: missing, F: filling, T: teeth) is a decisive factor for determination of the caries status (This index is based on clinical examination of individuals by the number of decayed, missing due to caries only and restored teeth).

The present study was undertaken to investigate the relation between *Lactobacillus* species in the human mouth and the DMFT index. Furthermore, the antibacterial effect of commercial toothpastes was assessed on the isolates to achieve information concerning to the effect of different toothpastes on oral microbiota.

2. Materials and Methods

2.1. Sample collection and DMFT index determination

A total, ninety swab samples were collected from saliva and tooth surfaces of the patient (male (50%) and female (50%)) attended to clinical dentistry in Shiraz, Iran.

In the present study decayed, missing, and filled teeth (DMFT) index for each person was determined by the dentists. DMFT index of each patient was measured based on DMFT scoring scale.

DMFT Score 1 to 4 = Low Caries status
DMFT Score 5 to 9 = Medium Caries Status
DMFT Score > 9 = High Caries Status

Then personal information concerning to sex, age, diet, and economic conditionswas collected by completing the questioners sheet by the patients.

Finally, each swab sample was placed in 2 ml of MRS broth and transferred to the laboratory for microbiological analysis. The tubes incubated at 37°C for 24 hrs, then a loop full of suspension was streaked on MRS agar and the plates were incubated at 37°C for 48 hrs. After this period, the plates were examined for isolation of lactic acid bacteria.

2.2. Phenotypic identification of isolates

The isolates with colony morphology similar to lactic acid bacteria were picked up and assessed by Gram staining and biochemical tests viz catalase, indole, nitrate reduction, production of gelatinase and fermentation of sugars such as glucose, galactose, fructose, mannose, pentose, arabinose, xylose,ribose, lactose, maltose, sucrose and manitol (Hoque *et al.*, 2010).

2.3. Authentication of lactic acid bacterial isolates

Identification of *Lactobacillus* isolates was verified by 16SrRNA gene sequencing. To perform the test, DNA of *Lactobacillus*isolates was extracted by DNA extraction Kit (Roche-Germany). Then, 16SrRNA gene of each isolates was amplified by Polymerase Chine Reactions (PCR). The primer used for amplification of 16SrRNA was F 5-CCA GCA GCC GCG GTA ATA CG-3 R: 5-ATC GGT ACC TTG TTA CGA CTT C-3. The purity of PCR products was evaluated by gel electrophoresis and the PCR products were sent to macrogen in South Korea (http://www.macrogen.com/) for DNA sequencing.

2.4. Bioinformatics applications

All sequences data were subjected to BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/) to definitively identify each respective 16S rRNA gene amplicon.

2.5. The relationship between the DMFT index and Lactobacillus spp. isolates

The relationship between *Lactobacillus* spp. isolated from the mouth and DMFT index was determined after identification of the isolates from each patient and DMFT index of the same.

2.6. Susecptibility of Lactobacillus spp. to commercial toothpasts

Five commercial toothpastes (tp1-tp5) were purchased from shops. One gram from each

toothpaste was added to 9ml of sterile distilled water. Isolated *Lactobacillus* was fully cultivated onto MRS medium, and the wells were made in the agar using sterile borel. Then, diluted toothpastes (10^{-1}) were added to the wells. The plates were incubated at 37°C for 48hrs. The zones of inhibition around the wells were considered as an antibacterial effect of the toothpastes.

3. Results

3.1. Isolation and identification of Lactobacillus spp.

Suspected colonies were grown on all cultivated MRS media. Phenotypic identification of the isolates recognized all of them as *Lactobacillus* spp. In addition, molecular identification of the isolated verified the isolation of different species of *Lactobacillus* (Figure 1 and Table 1).

3.2. Determination of DMFT index of the patients

The results obtained from the calculation of DMFT index have shown in Table 2. As seen in this table the mean of the DMFT index in young age group was relatively lower than middle and old age groups. The mean of DMFT index for male patients increased from 5.25 to 11.9 by increasing the age groups. The mean of DMFT index for female was relatively higher in middle age.

3.3. Relation between DMFT index and Lactobacillus spp. isolates

The results obtained from the relation between *Lactobacillus* isolates and DMFT score indicated that the frequency of occurrence of *L.plantarum*, *L.rhamnosus* and *L.mali* was high in the patients with low DMFT score. However, occurrence of *L.otakiensis*, *L.diolivorans* and *L.kefiri* was higher in the patients with high DMFT score.

3.4. Susceptibility of Lactobacillus spp. to commercial tooth pastes

The results obtained from the susceptibility of *Lactobacillus* spp. against commercial tooth pastes indicated that *Lactobacillus* species exhibited different reponses against commercial toothpastes. Among all the isolates *L. otakiensis* was resistant and *L. plantarum*, *L. rhamnosus* were sensitive to commercial toothpastes (Table 3).

4. Discussion

The oral microbial flora consists of several beneficial bacteria that are associated with oral health conditions. *Streptococcus* and *Lactobacillus* are the predominant bacteria in the oral cavity (Stamatova and Meurman, 2009). Although, these bacteria might play important role in the development of dental biofilms (Marsh and Devine 2011), some of them could control the progression of oral disease.

For instance, *Streptococcus thermophiles* and *Weissella cibaria* inhibit biofilm formation by *Streptococcus sobrinus* and *Streptococcus mutans* (Sinkiewicz et al., 2006). Mustafa and his colleguesin 2001 reported that the dental health of children related to their microflora. Hence, based on foregoing evidence oral health conditions depended on the type and population of oral microflora.

The present study investigated on the existence of different species of *Lactobacillus* in the oral cavity and their relation to DMFT index. The results obtained indicated that *L. plantarum, L. rhamnosus, L. mali L. otakiensis, L. diolivorans* and *L. kefiri* were isolated from the patients suffering from oral diseases. *L. plantarum*, *L. rhamnosus* and *L. Mali* was associated with the patients with a low DMFT score and *L. otakiensis, L. diolivorans* and *L. Kefir* was associated with the patients with a high DMFT score. It must be noted that oral microflora play two roles: benefit and detriment. It means some oral microflora related to oral disease and some could eliminate biofilm formation of oral pathogenic bacteria. Therefore, high population of beneficial bacteria in the oral cavity could increase oral health conditions. Diet and personal hygiene are two important factors for increasing oral health conditions (König, 2011). Dairy products contain *Lactobacillus* and *Streptococcus* could increase oral health conditions and decrease tooth decay (Saikali et al., 2004).

In this regards, regular consumption of yoghurt contain *Lactobacillus reuteri* decrease the population of *Streptococcus mutans* and decrease dental carries (Patil and Reddy, 2006).

Usually, consumption of toothpaste considered a special factor for managing the personal hygiene, however, the most of toothpastes decrease population of beneficial bacteria. Based on calculation of the DMFT index of the patient in the present study, personal hygiene of middle age was low however, consumption of toothpastes in them must be relatively more. Thus, it must be interpreted that consumption of toothpastes without adequate information concerning to their antimicrobial effect might have a side effect on beneficial oral microflora and increased the risk of oral diseases.

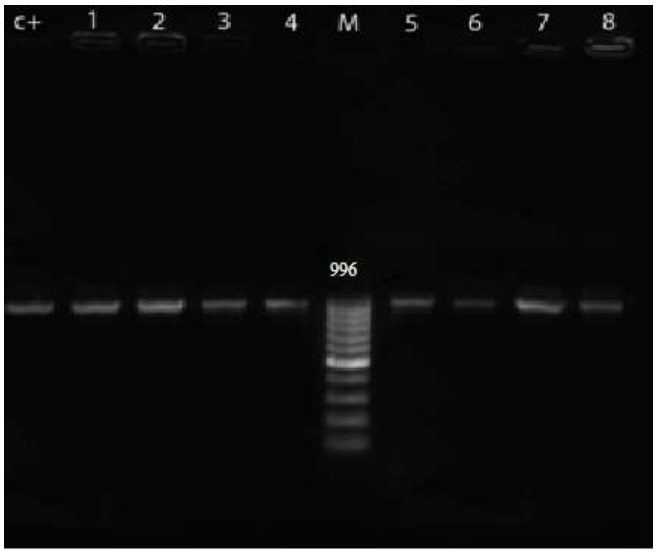

Figure 1. Gel electrophoresis of 16SrRNA genes of *Lactobacillus* spp. isolates. Lanes 1 to 8 are 16SrRNA (PCR product) of presumptive *Lactobacillus* spp. M – molecular weight marker FX174/BsuRI

Table 1.molecular identification of *Lactobacillus* spp. isolated from mouths

***Lactobacillus* strains**	**accession number**	**Identities**
Lactobacillus plantarum	gb|FJ749374.1|	99%
Lactobacillus rhamnosus	ref|NR_043408.1|	98%
Lactobacillus otakiensis	ref|NR_041657.1|	99%
Lactobacillus diolivorans	gb|KC571203.1|	99%
Lactobacillus kefiri	dbj|AB429371.1|	99%
Lactobacillus mali	ref|NR_112691.1|	99%

Table 2. DMFT score of the patients in different ages

The mean DMFT index for		
Age (year)	Male	Female
20<	5.25 (±0.15)	6.3 (±0.11)
20-45	9.9 (±0.22)	10.1(±0.27)
45<	11.9 (±0.31)	9.6 (±0.38)

Table 3. Susceptibility of *Lactobacillus* spp. to commercial toothpastes

inhibition zone (mm)of commercial toothpastes against						
Toothpastes	*L.plantarum*	*L.rhamnosus*	*L.mali*	*L. otakiensis*	*L.diolivorans*	*L.kefiri*
Tp1	13	14	15	8	14	12
Tp2	12	12	12	8	10	12
Tp3	12	11	8	10	11	13
Tp4	14	14	12	8	9	10
Tp5	13	13	11	7	11	13

Refereces

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