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Research Article

Identification of new Lactobacillus species that suppress tooth caries induced by salivary trace elements

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Abstract: The purpose of this study was to look into the relationship between Lactobacillus, which causes tooth decay, and the concentration of trace elements in saliva. A total of 100 samples were taken from patients with caries, whereas 11 samples were gathered from persons without caries as a control group. Only 26 *Lactobacillus* isolates were found in caries patients, including *L. fermentum*, *L. casei*, and a low proportion of *L. plantarum*. In the control group, the most common *Lactobacillus* isolates were *L. plantarum*, *L. terrae*, *L. letivazi*, and *L. helveticus*.Trace element concentrations varied across the study and control groups. The concentrations of Na, K, Zn and Cu were greater in the control group than in the study group, at significant variations (*p*-values = 0.02, 0.004, 0.021, and 0.002, respectively). However, there were no significant variations in (Pb) levels between the two groups. Increased amounts of Na, K, Ca, and Zn in cavity-free saliva may promote the growth of several *Lactobacillus* species that can protect against dental caries. Lower quantities of these trace elements in saliva, on the other hand, may promote the growth of other *Lactobacillus* strains linked to tooth decay.

Keywords: Dental caries, *Lactobacillus* spp., Trace elements

How to Cite:

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INTRODUCTION

A person's mouth is habitat to a diverse range of microbes, each with its own unique environment. These bacteria' contact and cooperation perform an important role in protecting the body from dangerous diseases. However, any change in the equilibrium of the oral microbiota can cause oral problems such as decay in the teeth and gum disease, as well as general illnesses (Gao et al., 2019). Multiple microbes are present in the oral microbiome, including *Streptococcus* , *Lactobacillus* and *Enterococcus* have been found as contributing factors to tooth caries (Bourgeois et al., 2017). These microbes can cling to the outer layer of teeth (Mattos-Graner et al.,2014). While some trace elements, such as fluoride, have been recognized for their preventative effects on tooth decay, the effect of additional trace elements on oral wellness is still not completely known. This is a topic of research and controversy (Watanabe et al ., 2011). The purpose of this study is to investigate The correlation between salivary trace elements, including copper and zinc, as well as the presence of lead, potassium, and sodium with specific strains of *Lactobacillus* spp. that are responsible for tooth decay and were obtained from individuals with dental caries, also their association with different strains of *Lactobacillus* found in individuals without dental issues in Thi-Qar Governorate.

METHOD

Samples of saliva and dental caries with dental plaque surrounding it were employed according (Abed et al., 2023). Only 58 saliva samples were obtain from dental caries patients and from 11 individuals without caries. Saliva samples were collected in the morning between 10:00 and 11:00 to ensure that the patients had not consumed any food or drinks prior to sample collection. Patients who had taken antibiotics within the two days leading up to the sample collection were excluded to avoid any potential impact of antibiotics on salivary composition and microbial profiles. Saliva samples that contained sputum or blood were also excluded. The presence of sputum or blood could interfere with the analysis and compromise the accuracy of the results. To stimulate saliva secretion, the patients were asked to chew gum Arabic for five minutes. Additionally, the patients were suggested to eat lemon.

Dental Caries and Dental Plaque Samples Collection

The samples of 100 patients were obtained from decayed tissue (pit and fissure cavity, smooth side cavity, and decayed root) and from plaque that is located on the border of the caries lesion area and is in contact with it. Samples a were selected implementing the following approach in which, The patient's oral cavity was rinsed with pure water many times ,also ,the tooth, especially the decayed area, was dried using an air syringe. Every patient's plaques were removed from the gum sulcus using a disposable gracey curette. Decayed roots Samples were obtained from the patient in the Endodontic branch, where touching with any periodontitis or pus, if found, was avoided, as did contact any area of the mouth cavity during the sample collection. When rotten teeth are pulled, they should be rinsed with saline to clean out saliva as well as blood, and then a sample is obtained from the region of the tooth that has the decay.

Lactobacillus Isolation and Identification

Traditional Identification

Every single specimen were cultivated in the BHI broth under anaerobic circumstances at 37°C for 24 hours. Afterward, tenfold serial dilutions were made. The diluted samples were then plated on De Man, Rogosa and Sharpe (Lactobacilli MRS broth, Lactobacilli MRS agar) with caco3 for discrimination colonies belonging to *Lactobacillus* spp., and HiCrome *Lactobacillus* Selective Agar Base to confirm the diagnosis of bacterial isolates then VITEK 2 system was used .

Molecular Diagnosis

For molecular diagnostic purposes, the provided primers were utilized to validate the identification of *Lactobacillus* spp (Kwon et al., 2004; Sukrama et al., 2012). The sequences of nucleotide were analyzed and digested utilising the NCBI-Blast Alignment recognition program located at :http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi

Table 1. 16s RNA primers

Primer	Sequence	Primer sequence	Size of Product bp
16s RNA	F	5'- AGAGTTTGATCCTGGCTCAG- 3'	1250
	R	5'- GGTTACCTTGTTACGACTT- 3’	
16S rRNA	F	5'CCACCTTCCTCCGGTTTGTCA '3	727bp
	R	5'AGGGTGAAGTCGTAACAAGTAGCC'3	

Most of *Lactobacillus* isolates have been sequenced and recorded in the GenBank

Trace Elements and Inorganic Salivary Constituents Analysis

Total of 5 ml whole saliva was collected from only 58 patients and from 11 individuals in control group once without repeating the sample collection. Immediately after collection, tubes were closed and transferred to the laboratory within one hour of collection. About 0.5 ml of HNO 3 was measured with the help of the pipette and added to the saliva to lower pH below 2 and prevent any microbial growth and enzymatic changes. Then the saliva was centrifuged at 4,000 rpm for 10 minutes (Banderas-Tarabay et al., 2002). The obtained sample was mixed with approximately 5 ml of deionized redistilled water. An aggregate of 10 milliliters of saliva was gathered in a test tube. The very adaptable dual beam Atomic absorption Spectrometer (Model - Varion AA575ABQ) was used to determine a precise percentage of inorganic and trace elements in saliva.

RESULT

In the study group, a collective number of thirteen *L.fermentum* isolates (equivalent to 13% of the patients) were identified and isolated. Alongside, there were seven *L.casei* isolates (7% of the patients) and six *L.plantarum* isolates (6% of the patients) that were also isolated and confirmed. Regarding the control group, a total of eight *L. plantarum* isolates were found (making up 72.7% of the individuals). In addition, there were two isolates each of *L. letivazi* and *L. helveticus* (constituting 18.1% of the individuals), while one isolate of *L. terrae* was discovered (also accounting for 9.1% of the individuals). When cultured on Lactobacilli MRS agar, the *Lactobacillus* colonies exhibited varying sizes, with some being small and others being large. These colonies appeared pale or cream colored, had a convex and smooth shape, and were surrounded by a transparent halo, as depicted in Figure 1. Confirmation tests were carried out using the VITEK2 compact for *Lactobacillus* as shown in Figure 3.

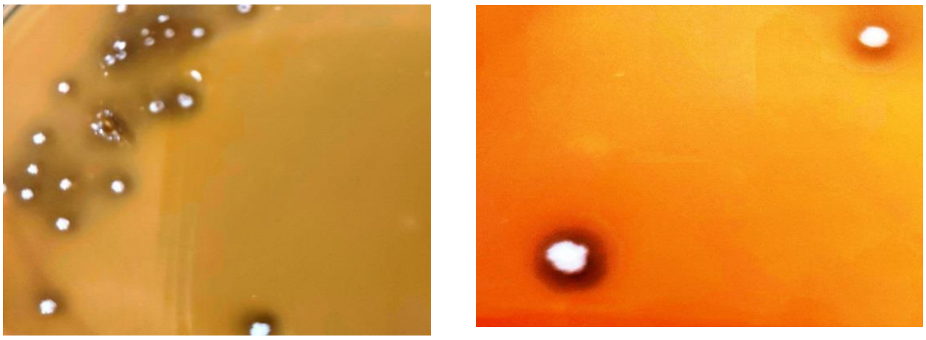
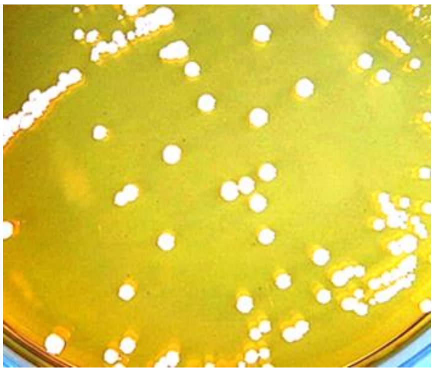
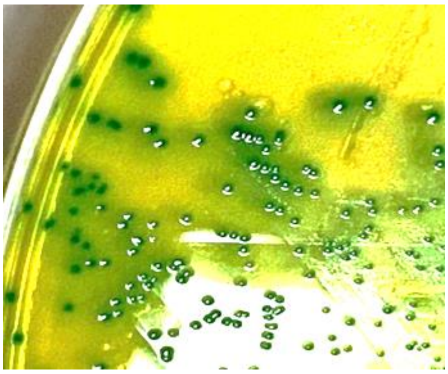
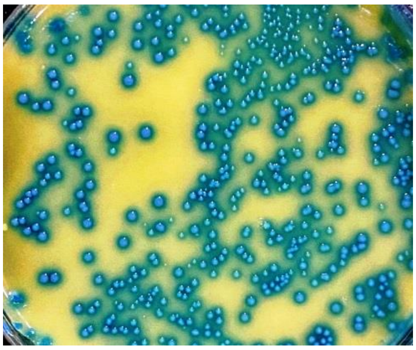


Figure 1: Colonies of different *Lactobacillus* colonies on Lactobacilli MRS agar





L.fermentum L.casei



L.plantarum

Figure 2. Colonies of lactobacillus on HiCrome Lactobacillus Selective Agar Base

Genomic DNA extraction Kit was utilized to extract DNA from the sample isolates. The DNA was visually Radial gel electrophoresis utilizing a gel composed of 1% agarose was performed before and following PCR amplification of the 16.S.r.RNA gene Figure 4.

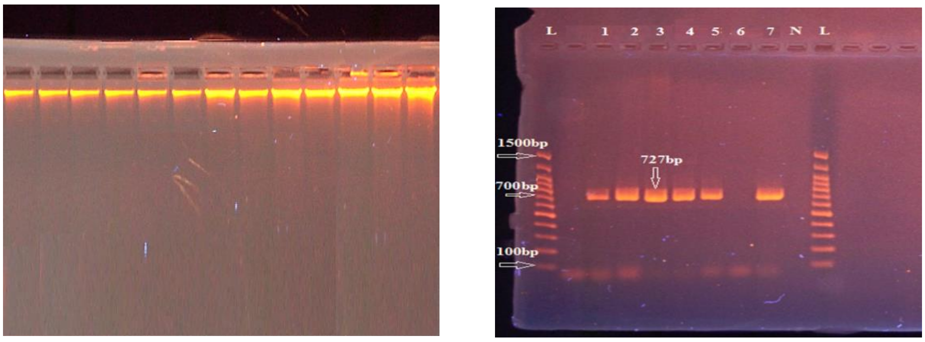


Figure 3. Gel electrophoresis before and after PCR product

Table 1. Accession numbers of *Lactobacillus* spp.

***Lactobacillus* isolates**	**Accession numbers**
L.plantarim	**OQ933116**
L.plantarim	**OQ933117**
L.plantarim	**OQ933118**
L.plantarim	**OQ933119**
L.plantarim	**OQ933107**
L.plantarim	**OQ933108**
L.terrae	**OQ933121**
L.fermentum	**OQ933109**
L.fermentum	**OQ933110**
L.fermentum	**OQ933120**
L.letivazi	**OQ933122**
L.letivazi	**OQ933123**
L.helviticus	**OQ933111**
L.helviticus	**OQ933112**

Trace Elements and Inorganic Salivary Constituents Analysis Result

Table 2 display the values of trace elements (Zn, Cu, and Pb) as well as inorganic salivary constituents (Na and K) for study groups. The data includes mean and standard deviation for 58 patients diagnosed with dental caries these patients were included 27 youngster, 22 Adolescent, and 9 elderly individuals. Results showed that the total sample, of the salivary sodium ions were found to be higher in the Adolescent compared to youngster and elderly with mean value (123.8277 mg/l). Also, the elderly had the highest average value of salivary potassium, which was 319.6544 mg/l. On the other hand, the adolescent group had the highest percentage of zinc, which was 0.0595 mg/l with a significant difference in the levels of these two ions compared to the rest of the categories. All results have SIG. 0.002.

Table 2. Values of the patients trace elements and inorganic salivary constituents

Item		Na mg/l	K mg/l	Zn mg/l	Cu mg/l	Pb mg/l
youngster	Mean	57.4826 b	87.5033 b	.0073 a	.0138 b	.0017 b
	N	27	27	27	27	27
	Std. Deviation	31.59819	44.14492	.01034	.01682	.00184
Adolescent	Mean	123.8277 a	226.0095 a	.0195 a	.0143 b	.0010 b
	N	22	22	22	22	22
	Std. Deviation	92.41819	180.38048	.02905	.01837	.00182
elders	Mean	76.5000 b	319.6544 a	.0176 a	.0595 a	.0507 a
	N	9	9	9	9	9
	Std. Deviation	26.93395	202.29689	.02437	.13507	.06536
Total	Mean	85.5990	176.0636	.0135	.0211	.0090
	N	58	58	58	58	58
	Std. Deviation	68.23177	162.95220	.02186	.05559	.03045

The Table 3 presented provides a comparison of the salivary trace elements and inorganic ions present in the youngster individuals with dental caries, and those without caries, who serve as the control group. According to the findings, there was significant variation between the study group and the control group with respect to sodium, potassium, and zinc levels, whereas there was no significant difference observed in lead levels. According to the findings, there was significant variation (Sig. less than 0.01) between the study group and the control group with regard to sodium, potassium, and zinc levels, whereas there was no significant difference observed in lead levels. The study revealed that the levels of sodium, potassium, and zinc (57.4826, 87.5033, and 0.0073, respectively) were comparatively reduced in the study group as compared to the control group (140.24, 288.72, and .2926, respectively) .

Table 3. Comparison of the Na, K, Zn and Pb concentration value of the study and control groups in the youth group

		N	Mean	t-value	sig	Std. Deviation	Std. Error Mean
Na	young	27	57.4826	-3.952-	.000	31.59819	6.08107
	control	11	1.4024E2	-2.724-	.020	98.72502	29.76671
K	young	27	87.5033	-5.492-	.000	44.14492	8.49569
	control	11	2.8872E2	-3.647-	.004	180.83174	54.52282
Zn	young	27	.0073	-4.370-	.000	.01034	.00199
	control	11	.2926	-2.735-	.021	.34596	.10431
Cu	young	27	.0138	-3.358-	.002	.01682	.00324
	control	11	.0384	-2.736-	.017	.02780	.00838
Pb	young	27	.0017	1.044	.304	.00184	.00035
	control	11	.0010	1.134	.269	.00151	.00046

DISCUSSION

A significant presence of diverse *Lactobacillus* species was observed in individuals suffering from dental caries. These specific *Lactobacillus* species were found to be associated with the proportions of trace elements present in the saliva of caries patients. Conversely, different species of Lactobacillus were discovered in the mouths of healthy young individuals, showing elevated concentrations of trace elements in their saliva. *Lactobacillus* is considered to be the next most cariogenic bacterium in the oral microbiome. Farthermore, it is not the main reason of decay, it serves an important part in lesions progression. (Ademe et al ., 2020). In our study, total of 26 *Lactobacillus* isolates were identified, with 13 of them being *L. fermentum*, 7 *L. casei*, and 6 *L. plantarum*. These findings align with the research of (Lapirattanakul et al., 2020), which identified *L. fermentum* as the most frequently isolated *Lactobacillus* from children with caries by followed by *L. casei*.In our study, *L. plantarum* was identified as being less common than other *Lactobacillus* in carious lesions. Only five isolates were detected in adolescents with caries, and just one isolate was found in young adults. These findings are in compliance with the results of (Ahirwar et al., 2019), who also reported a lower prevalence of *L. plantarum* in dental caries cases. The most prevalent types of bacteria found in both adults and pediatric dental caries, including particularly severe young child cavities, are *L. casei* and *L. fermentum* (Caufield et al ., 2015). The control group had a limited number (only 11) because it was difficult to find people who did not have tooth decay or without any history of tooth decay because it is a prevalent disease affecting nearly all adults globally, as well as 90% of children , this facts were confirmed by Kale et al., (2019) . *Lactobacillus* was isolated and characterized from control group differ from the ones isolated and diagnosed in individuals with dental caries. The current study results reveal that certain species of *Lactobacillus* may have a preventive involvement in the incident of caries in the control group of young participants, while other species may contribute to the development of caries. Specifically, *L. fermentum* and *L.casei* were found at higher rates in carious lesions of patients with caries compared to *L. plantarum*, which was present at a lower rate in patients but at a higher percentage in the control group. According to the researchers (Zeng et al., 2022) who concluded that out of all the probiotic species that were tested, *L. plantarum* displayed excellent ability to prevent the growth of *C. albicans* and *S. mutans*, interfere with the formation of harmful biofilms by inhibiting growth of them with their exopolysaccharides. This supports the results of the current study in which *L.plantarum* was dominant in the saliva of people without caries, thus preventing the growth or reducing the effect of *S.mutans* and preventing the growth of *C. albicans* in the control group. In the recent study, *L. terrae* was isolated and diagnosed in the saliva of (control) individuals without dental caries, marking the first time this particular strain has been isolated from oral cavity in both Iraq and globally. Previous research conducted by (Kim et al., 2018) had identified *L. terrae* in soil samples. In the current study, for the first time in Iraq, *L. letivazi* has been isolated and identified in the oral cavity of individuals who do not have tooth decay. This finding is consistent with previous studies of Anisha Deborah, (2018) where the same bacteria was found in healthy individuals without periodontal or any oral diseases. (Kobatake et al., 2019) proved the capability of *L. helveticus* to inhibit some pathogenic bacteria in the oral cavity and subsequently employed *L. helveticus* in laboratory tests to lessen the quantity of *P. gingivalis*, that cause periodontal disease. The existence of *L.plantarum* ,*L. letivazi* ,*L helveticus* and *L.terrae* in the saliva of individuals who do not suffer from tooth decay. This can result in the non-growth of *S. mutans*, *C. albicans*, and *E. faecalis*, which are lead to do a significant role in causing dental caries. or maybe prevent the formation of biofilm of them , which could further aid in preventing the development of dental caries especially in adults category. The study involved 100 patients, but only 58 samples were obtained due to various reasons such as the difficulty of obtaining saliva from patients experiencing pain or insufficient amounts (less than 5 ml). In some cases, it was also challenging to obtain parental consent, especially when dealing with children. Among the people who suffer from caries, the mean value of sodium showed higher in adolescents (123.8277) than its value in young people and the elderly with clear statistical significance, while the mean value of potassium (87.5033) in young people was less than its value in adolescents and the elderly. It was also noted that the average value of copper and lead increased in the elderly (.0595, .0507), respectively, higher than their values in adolescents and young adults. There was no significant difference in the mean value of zinc among the study groups. However, the study revealed that the average levels of sodium, potassium, copper, and zinc were significantly higher (with a significance level less than 0.01) in the control group compared to the study group. In contrast, there was no significant difference observed in lead concentration between the two groups. The findings of the present research align with the study carried out by (Anandan el al., 2022), which concluded that individuals who are free from dental caries have a higher salivary sodium percentage compared to those who have tooth decay. According to research of Banoosh, (2015), the control group had a higher concentration of sodium and potassium in their saliva when compared to the study group (individuals with tooth decay). However, the disparity was not statistically significant. In contrast, our recent study has demonstrated a significant rise in the ratio of sodium and potassium in the control section versus the research group. In research of (Paradeshi et al ., 2018), copper has an impact on the ability of *L. helveticus* to withstand acidity and salt, and increases its susceptibility to the production of antimicrobials but this study had done on cheese. Drawing a comparison between his research and our current study, it was noted that individuals with higher concentrations of copper in their saliva, including those with dental caries, may stimulate probiotics to combat caries-causing bacteria more efficiently than those with lower copper levels. copper may serves a part in the prevent occurrence of dental caries that observed in such individuals. In our current study, particular of *Lactobacillus* were diagnosed in the control group, which is composed of individuals without dental caries. This is distinct from other *Lactobacillus* that were diagnosed in the study groups patients with caries. Moreover, a link between trace elements, particularly Zn and Cu, has been demonstrated, as there are considerable differences in their amounts across both categories. *L.plantarum* is recognized to have antibacterial and antioxidant qualities. Furthermore, it has been able of generating bacteriocins (Garcia-Gonzalez, 2021). *L.plantarum* has been found in just a few percent of persons with caries, but in greater proportions among those with no caries. This finding can be related to the greater concentration of Cu and Zn discovered in the group serving as the control, which may have lowered the organism's capacity to induce acidity while simultaneously improving its antibacterial potential in control group. (Meng et al., 2022) revealed that the presence of Zn increases growth rate of *L. plantarum*. This resulted in an advantageous impact upon the physiological activities of *L. plantarum*, The zinc raised strain had a more tolerance for acid, bile salt, and H_2O_2. Furthermore, the addition of zinc improved the strain's antioxidant capabilities, including as its elimination of radicals capability and the enzyme's anti-oxidative activity.

CONCLUSION

It appears that people without caries have higher concentrations of Na, K, Cu, and Zn in their saliva, which could encourage the growth of various Lactobacillus species that may prevent dental caries, whereas lower concentrations of the same salivary trace elements promote the growth of different Lactobacillus spp. that lead to caries.

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