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Molecular detection of *Lactobacillus acidophilus* in the dental plaque of Iraqi patients

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ABSTRACT

Microbial colonization of both hard and soft oral surfaces constitutes the primary component of dental plaque. The probiotic strain *Lactobacillus acidophilus* has been implicated in the formation of dental plaque, with its heightened acidogenicity considered a potential adverse effect. This study aimed to investigate the presence of *L. acidophilus* within the oral biofilm of periodontally healthy individuals. A total of 90 subjects aged 20–40 years were enrolled. Specimens were collected from patients attending a private dental clinic in Hillah, Iraq, seeking comprehensive dental and periodontal evaluation between October 2022 and February 2023. Both male and female participants were included. The detection of *L. acidophilus* was performed using polymerase chain reaction (PCR) and conventional culturing methods. Among the 42 samples analysed *via* culture, only 2 (4.8%) tested positive. In contrast, PCR identified *L. acidophilus* in 4 out of 48 samples (8.3%). Although PCR is widely regarded as the most sensitive and specific method for bacterial identification, its accuracy may be compromised by technical variables such as excessive sample dilution.

1. Introduction

Lactobacillus acidophilus is a non-motile, Gram-positive bacterium that exhibits either rod-shaped or coccoid morphology. As a terminal product of its metabolic and fermentative activity, *L. acidophilus* produces lactic acid. This species can survive in environments with a pH as low as 4–5, or even lower[1]. *L. acidophilus* was the first bacterial species to be classified as a probiotic. Among oral probiotics, *Lactobacillus* spp. are potent inhibitors of key periodontal pathogens, in-

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cluding *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Prevotella intermedia*. Due to their highly aciduric and acidogenic nature, *Lactobacillus* spp. are predominantly found in deep carious lesions[2].

Compared to the diverse array of pathogenic and commensal bacteria that colonize oral surfaces in biofilm form, *Lactobacillus* spp. play a secondary role in lesion progression and biofilm composition, rather than in lesion initiation. Notably, the population of *Streptococcus mutans* declines as pH levels drop, while *Lactobacillus* spp. increase in number under acidic conditions[3,4]. Like *S. mutans*, *L. acidophilus* is considered part of the normal oral flora. However, its abundance increases with the advancement of carious lesions[5]. In highly acidic environments (such as deep cavities) *L. acidophilus* reaches peak concentrations and is thus regarded as a secondary invader[6].

In the context of periodontal health, *L. acidophilus* exhibits inhibitory effects against *P. gingivalis* by downregulating proinflammatory interleukins, including interleukin (IL)-6, IL-1, and IL-8, which are induced by this pathogen[7]. Additionally, *L. acidophilus* can co-aggregate with *Fusobacterium nucleatum*, thereby preventing its adhesion and invasion of host tissues[8]. *L. acidophilus* also demonstrates bactericidal activity against *A. actinomycetemcomitans* through the production of specific enzymes, such as lipase[9]. Given these properties, *L. acidophilus* is considered a promising periodontal probiotic with potential future applications in the prevention of periodontal diseases[10].

The present study aimed at detecting *L. acidophilus* using both conventional culturing and polymerase chain reaction (PCR) techniques. It also sought to evaluate the effects of chlorhexidine (CHX) and green tea oil on *L. acidophilus* viability.

2. Methodology

Ninety dental plaque samples were collected from patients attending a private dental clinic in Hillah, Iraq. Swabs were taken from plaque deposits of individuals aged 20–40 years. Subjects undergoing pharmacological treatment were excluded. Sampling occurred between October 2022 and February 2023. Ethical approval was obtained from the College of Dentistry of the University of Babylon (approval number: 70; date: 26/2/2025).

Growth and colonization of *Lactobacillus* spp. were supported using the de Man, Rogosa and Sharpe (MRS) agar. After 24 h of anaerobic incubation at 37°C, the characteristic colony morphology was assessed in order to identify the target bacteria.

For molecular detection, *L. acidophilus* was identified *via* PCR. Approximately 780 base pairs (bp) of *Lactobacillus* DNA were amplified by using primers targeting the 16S ribosomal RNA (rRNA) gene. DNA was extracted from biofilm samples that were collected *via* sterile swabs, which were immediately placed in anaerobic transport vials or sterile tubes. PCR products were analysed by gel electrophoresis.

3. Results and Discussion

Of the 90 dental plaque samples analysed, only 2 (4.8%) out of 42 were found positive for *L. acidophilus* using the culturing method. In contrast, PCR detected *L. acidophilus* in 4 (8.3%) out of 48 samples, as illustrated in Figure 1. *L. acidophilus* was identified by PCR through amplification of the 16S rRNA gene using specific primers. The presence of two distinct bands confirmed the detection of *L. acidophilus*.

Several factors may account for the low detection rate, including low bacterial concentration in highly diluted samples and potential mutations in the amplified DNA fragment due to polymerase error. Primer non-specificity may also lead to off-target amplification, compromising PCR specificity. Furthermore, intra-species variability in colonization capacity has been documented for *Lactobacillus* spp. Despite these limitations, 16S rRNA gene sequencing remains a powerful tool for microbial identification, including novel species. PCR continues to be a relevant and sensitive technique for routine microbial diagnostics.

The present study has also demonstrated that CHX (0.2%) exhibited the highest inhibitory activity against *L. acidophilus*. This effect is attributed to

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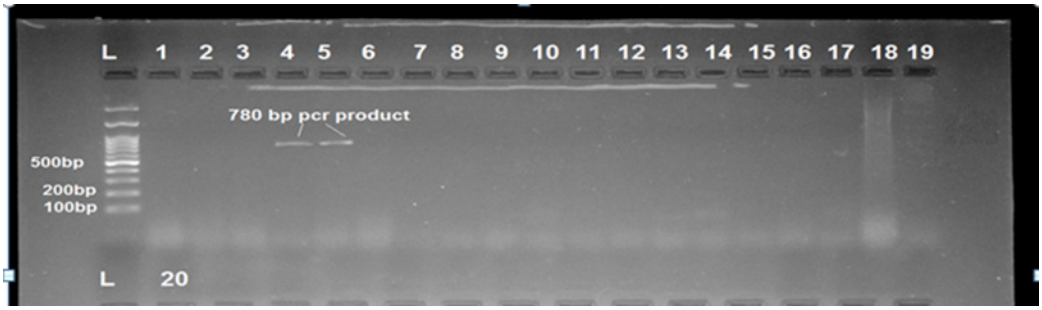

Figure 1. *Electrophoresis of 1.5% agarose gel for 60 min at 72 V, showing the polymerase chain reaction (PCR) amplification of Lactobacillus acidophilus (780 bp). Lanes 4–5 represent positive isolates; lane M denotes the 100-bp DNA marker.*

CHX's potent antimicrobial properties, which disrupt microbial cell membranes and increase intracellular leakage. Conversely, green tea oil showed comparatively lower inhibition of *L. acidophilus*. Although antimicrobial oils can affect microbial activity, their efficacy varies depending on the chemical composition and ability to penetrate bacterial structures.

4. Conclusion

PCR is regarded as the most accurate method for bacterial detection due to its high sensitivity and specificity. However, its performance may be affected by factors such as excessive sample dilution and cross-reactivity.

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Conflicts of interest

None exist.

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