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Short Communication

Antimicrobial activity of *Lactobacillus plantarum* against oral microbial plaque

Mansour Amin[1*], Farzane shafiee[2] and Neda Rasaie[2]

[1]Department of Microbiology, school of medicine, Infectious and tropical diseases research center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[2]Department of periodontology, School of Dentistry, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

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Some strains of lactic acid bacteria have a favorable influence on physiologic and pathological processes of the host due to their specific health-promoting probiotic characteristics that relate to modulation of the immune system. The aim of the present study is to evaluate the possibility of antimicrobial substances against oral microbial plaque, extracted from *Lactobacillus plantarum* during its growth in broth culture media. 50 samples collected from white headed cabbage and kept in sterile tubes containing Man-Rogosa-Sharpe medium (MRS) broth were incubated for 3 days, and then subcultured on MRS agar. The grown colonies were characterized by phenotypical properties. Polymerase chain reaction (PCR) analyses were then performed on the extracted DNA from cultures. The colony of *L. plantarum* confirmed by PCR, were inoculated in MRS broth for 5 days and then mixed with ethyle acetate. The solution was separated into two phases, in which the supernatant comprised of the extracted antimicrobial compound. The supernatant was then dried at 45°C and was used for antimicrobial susceptibility by E. Test. The MIC's of this compound which affected target bacteria were as follow: *Streptococcus mutans* 0.1 mg/ml, *Streptococcus salivarius* 0.05 mg/ml, *Streptococcus sanguis* 0.2 mg/ml, and *Lactobacillus casei* 0.05 mg/ml. According to this study and others, *L. plantarum* can produce antimicrobial compounds and these bacteria exist in fresh vegetables; consumption of such vegetables may colonize this probiotic and other useful probiotics in the mouth and intestines and protect these parts of body from pathogens.

Key words: *Lactobacillus plantarum,* oral plaque and antimicrobial.

INTRODUCTION

Many definitions of probiotics have been published, starting from Fuller, who defined a probiotics as a live microbial feed supplement, which beneficially affects the host by improving its intestinal microbial balance (Fuller, 1989). The genera most commonly used in probiotic preparation are *Lactobacillus, Bifidobacterium, Streptococcus, Lactococcus* and some fungal strains (Galdeano et al., 2007). This genus of bacteria is widely distributed in the environment. Several species, including *lactobacillus acidophilus*, are members of the normal intestinal and vaginal flora of healthy humans. Other species, such as *Lactobacillus bulgaricus* and *Lactobacillus casei*, are commonly isolated from dairy products as well as from fruits and vegetables and they play an important role as probiotics in human and animal nutrition (Oyetayo, 2004; Erdogrul 2006). There is evidence that some strains of lactic acid bacteria have a favorable influence on physiologic and pathological processes of the host due to their specific health-promoting probiotic characteristics that relate to modulation of the immune system (Beatriz et al., 2008). The genus *Lactobacillus* contains over 110 species which are classified in three major groups: the obligate homofermentative lactobacilli which ferment hexoses to lactic acid; the facultative heterofermentative lactobacilli, which ferment haxoses to lactic acid only or to lactic acid together with acetic acid, ethanol and formic acid under glucose limitation; and obligate heterofermentative

*Corresponding author. E-mail: mnsamin@yahoo.com.

lactobacilli, which ferment hexoses to lactic acid, acetic acid, ethanol and CO_2 and ferment pentoses to lactic acid and acetic acid (Amin et al., 2009).

The aim of the present study is to evaluate the possibility of antimicrobial substances against oral microbial plaque, extracted from *L. plantarum* during its growth in broth culture media.

MATERIALS AND METHODS

Sample collection and culture

50 samples collected by wet swabs from white headed cabbage were kept in sterile tubes containing MRS broth media in summer season. The entire sample tubes where incubated at 37°C and 5% CO_2 conditions for 3 days, then subcultured on Man-Rogosa-Sharpe medium (MRS) agar (Hi-media, India) at mentioned conditions for 48 h. The grown colonies were characterized by phenotypical properties including morphology, gram positive staining, resistance to vancomycin and absence of catalase, oxidase and motility (Rodas et al., 2005; Forbes et al., 2007).

Polymerase chain reaction (PCR) analyses

PCR analyses were then performed on the extracted DNA from Stock cultures and were stored at -70°C in skim milk. 1 to 2 loops of the confirmed bacteria with phenotypically analysis grown on MRS agar were resolved in TE (Trace EDTA) buffer and boiled at 100°C for 15 min. Extracted DNA was selected for PCR analyses by using genus *Lactobacillus* primers (forward: 5-GGGTTCCCCATTCGGA-3, 560-640 bp and reverse: 5-GAATCGCTAGTAAATTCG-3) and species-specific primers (*L. plantarum* forward: 5-TCGGGATTACCAAACATCAC-3, 319 bp and Reverse: 5-CCGTTTATGCGGAACACCTA-3) (Massi et al., 2004).

The PCR conditions were initial denaturation of 94°C for 5 min followed by 30 cycle of denaturation at 94°C for 30s, annealing at 52°C for 30s for cas-ITS genus specific primers; 45°C for 45s for *L. plantarum*; 40°C for 40s for *L. casei* and final extention at 72°C for 10 min using a termocycler (eppendorf). The PCR products were analyzed on 1% agaros gel.

Extraction of antimicrobial substance

The colony of *L. plantarum* confirmed by PCR, inoculated in MRS broth. After 5 days Incubation, the MRS broth media containing bacteria was mixed with ethyl acetate (75:25) and agitated with a magnatic stirrer for 24 h. Then the media was allowed to settle for 30 min. following settlement, the solution was separated into two phases, which the supernatant was comprised of the extracted antimicrobial compound. The color of ethyl acetate was turned yellow after agitation. The supernatant was then dried at 45°C and was used for antimicrobial susceptibility test.

Antimicrobial susceptibility test

The dried extract was dissolved in methanol and its pH was adjusted to 7 using NaOH. The Minimal Inhibitory Concentration (MIC) of this antimicrobial substance determined using modified E. Test, by incorporating 20 µl of the each dilution of extract in paper discs and exposed against target bacteria (Amin and Kapadnis, 2005).

Target oral bacteria

The target bacteria which were used in this study were *Streptococcus mutans* (PTCC1683), *Streptococcus salivarius* (PTCC1448), *Streptococcus sanguis* (PTCC1449), and *L. casei* (PTCC1608), obtained from collection center of fungi and bacteria, Tehran, Iran. The experiments were repeated 3 times and the results were constant in all tests.

RESULT

The antimicrobial compound extracted from *L. plantarum* showed an effective antibacterial activity against all tested bacteria. The MIC's of this compound which affected target bacteria were as follow: *S. mutans* 0.1 mg/ml, *S. salivarius* 0.05 mg/ml, *S. sanguis* 0.2 mg/ml, *L. casei* 0.05 mg/ml (Table 1 and Figure 1).

DISCUSSION

Dental caries is caused by a specific group of cariogenic bacteria, like *S. mutans*, which convert dietary sugars into acids that dissolve the mineral in tooth structure. Killing cariogenic bacteria is an effective way to control or prevent tooth decay (Chu-Hong et al., 2011).

Recent reviews indicated that different lactobacillus strains may be isolated from various vegetables and fermented food including cheese, yoghurt, corn slurry, fruits, pounded yam and rice. The isolated bacteria showed antimicrobial activity in different values (Oyetayo, 2004; Erdogrul and Coolborn, 2005). *Lactobacillus* is a part of oral normal flora, contained antimicrobial substance that has inhibitory effect on growth of oral pathogens.

There are evidences that lactobacilli can inhibit the growth and attachment of pathogens to epithelial cells.

The hydrogen peroxide and bacteriocin-like compounds produced by lactobacilli can kill the pathogenic micro-organisms in human body (Falagas et al, 2006; Atassi et al, 2006). In the present study the extraction of *L. plantarum* was isolated and the organic acids which are produced by these bacteria were neutralized. In this part of the study, the antimicrobial compound had good effect against target bacteria. In the next part of the study, the obtained substance was autoclaved for 10 min for denaturalize of bacteriocin- like compounds, and then its antimicrobial activity again was evaluated. There was no significant change in MIC values before and after autoclave. With these evidences, we can say this antimicrobial substance is an antibiotic.

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Table 1. MIC values of antimicrobial substance extracted from *L. plantarum* against oral bacteria.

Microorganisms	MIC values (mg/ml)
S. mutans	0.1
S. salivarius	0.05
S. sanguis	0.2
L. casei	0.05

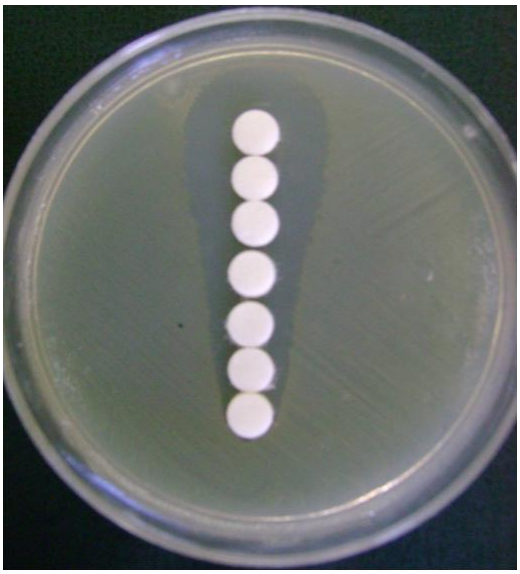

Figure 1. MIC of antimicrobial substance obtained from *L. plantarum* against *S. mutans* (E. Test).

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