

Isolation of lactic acid bacteria from fermented milk products and their antimicrobial activity against *Staphylococcus aureus*

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ABSTRACT

A total of 13 isolates of lactic acid bacteria (LAB) were isolated from curd and cheese samples. The samples were collected from the different regions of the Shiraz city. The isolates were identified as *Lactococcus* sps. (23%), *Pediococcus* sps.(54%), *Lactoba cillus* sps. (15%) and *Leuconostoc* sps. (18%) by biochemical characterization. These isolates were tested for their antimicrobial activity against Standard *S.aureus* by well diffusion method and MIC of all these isolates were observed to check the sensitivity of each isolate. Effect of LAB on the *S.aureus* was studied by SDS-PAGE.

Keywords: Lactic acid bacteria (LAB), Antimicrobial activity, MIC, *Staphylococcus aureus*.

INTRODUCTION

Lactic acid bacteria (LAB) are a group of related bacteria that produce lactic acid as a result of carbohydrate fermentation. They comprise a clade of Gram positive, usually non motile acid tolerant microorganisms. They are generally non-spore forming, non-respiring cocci, coccobacilli or rods. LAB growth lowers both the carbohydrate content of the food that they ferment and the pH due to the lactic acid production. Certain LAB strains have been reported to be highly antagonistic to biofilm forming *S.aureus* [1].

LAB strains are potentially promising because they generate bactericidal bioactive peptides (bacteriocins) and enzymes that are able to control biofilm formation and growth of pathogens [2].

LAB exerts strong antagonistic activity against many microorganisms, including food spoilage organism and pathogens. Some strains may contribute to food preservation of fermented food by producing bacteriocins [3]. The major parameters involved in bacterial growth inhibition are the pH, which decreases by the production of organic acid, nutrient competition, hydrogen peroxide and antibiotic production. *S.aureus* is a Gram positive coccus, non-motile non-spore forming facultative anaerobic which appears as grape like clusters. It is a common pathogen associated with hospital acquired diseases which causes major problem for public health. One of the major causes of staphylococcal enterotoxin is vomiting and diarrhea when ingested and is responsible for staphylococcal food poisoning [4].

MATERIALS AND METHODS

Microorganism

The pathogen namely *Staphylococcus aureus* was procured from Institute of Microbial Technology, Shiraz and LAB was isolated from samples of fermented milk products of curd and cheese collected from various regions of Shiraz

city. Identification of Lactic acid bacteria MRS Agar and MRS broth were used for enumeration and culture of Lactic acid bacteria at 37°C for 24 hrs. The cultures were identified by performing various morphological and biochemical characterization. It includes Gram reaction, Spore formation, Glucose production, hot loop test and Sugar fermentation test. After confirmation of LAB isolates, turbidity were matched with the McFarland standard series. Out of 13 isolates, 6 isolates were having 15×10^8 CFU/ml and 7 isolates were having 12×10^8 CFU/ml.

Determination of Antimicrobial Activity of LAB isolates against *S.aureus*

The antimicrobial activity of isolated LAB *S.aureus* was performed by well-diffusion method[5]. *S.aureus* was incubated in BHI broth at 37° C at 24 hrs. 15ml of Muller Hinton Agar was prepared and 150µl of *S.aureus* culture having 6×10^8 CFU/ml was inoculated into it. Once solidified the plates, wells of 6mm diameter were made and 40µl of each concentration of LAB isolates were filled into well [6]. Then the plates were incubated at 37°C for 24 hrs. And antimicrobial activity was determined by measuring the clear zone around the well [7].

RESULTS AND DISCUSSION

A total of 13 LAB isolates, 3 (23%) belonged to *Lactococcus*, 7 (54%) to *Pediococcus*, 2 (15%) to *Lactobacillus*, 1 (8%) to *Leuconostoc*, which has shown in figure 4. The antimicrobial activity of LAB isolates at different dilutions was tested against *S.aureus* which has shown in figure 5 and figure 6. Out of 6 isolates of LAB having 15×10^8 CFU/ml, S1 gave largest zone of inhibition followed by S13 (*Pediococcus*), S2 (*Pediococcus*) and S7 (*Pediococcus*) at 6×10^7 CFU/ml, while only 3 isolates S1(*Lactococcus*), S2 (*Pediococcus*) and S13 (*Pediococcus*) also gave inhibitory zones at 2×10^7 CFU/ml and 0.66×10^7 CFU/ml. On the other hand, out of 7 LAB isolates having 12×10^8 CFU/ml, S5 (*Leuconostoc*) and S8 (*Pediococcus*) gave largest zone of inhibition at 4.8×10^7 CFU/ml. These zones are followed by S12 (*Homofermentative Lactobacillus*) only gave zone of inhibition at 0.53×10^7 CFU/ml. These zones are followed by S12 (*Homofermentative Lactobacillus*), S10 (*Pediococcus*) S11 (*Pediococcus*) and S9 (*Pediococcus*).While S12 (*Homofermentative Lactobacillus*) only gave zone of inhibition at 0.53×10^7 CFU/ml. These results may be because of the wide spectrum of antimicrobial activities of LAB isolates which varies according to the serotype[6]. The results depicted that inhibition zones decreases as the concentration of LAB isolates. Out of the 13 isolates, S1 (*Lactococcus*), S2 (*Pediococcus*), S13 (*Pediococcus*) and S12 (*Lactobacillus*) gave lowest minimum inhibitory concentration of 0.66×10^7 CFU/ml and 0.53×10^7 CFU/ml. These 4 LAB isolates indicate that they are highly sensitive in inhibiting the growth of *S.aureus* even at a low concentration.

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