World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:6, No:1, 2012

Antibacterial Activity of Lactic Acid Bacteria Isolated from Table Olives against Skin Pathogens

M. Shafighi, Z. Emami, M. Shahsanaei, and E. Khaliliyan

***Abstract*—**The aim of this study was to assess the effect of LAB isolated from Iranian native olives on the opportunistic skin pathogens, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Lactic Acid Bacteria were isolated from the brine of each sample in the prior of time. The samples were spread on MRS agar for isolation of lactobacillus and for lactococcus. 28 strains of labs were isolated. The labs were centrifuged, the supernatant was strewed and pellet was used to inoculation in wells or at blank disks. 20µl of each pellet was inoculated to blank disks and 40µl of each pellet was inoculated to each well. The result of disk and well diffusion agar against these pathogens were confirmed each other. The size of inhibition zone was different according to the type of bacteria, the method and the concentrations of labs.

***Keywords*—**Olive, Probiotic, Lactic Acid Bacteria (LAB), *P.aeroginosa* and *S.aureus*

I. Introduction

PSEUDOMONAS *aeruginosa* is a gram-negative, aerobic, bacilli bacterium that can found in soil, water and skin flora[1].This bacterium is an opportunistic human pathogen. That caused nosocomial disease in immunocompromised individuals and typically infects the pulmonary tract, urinary tract, burns and wounds and also causes other blood infection.

It is the most frequent cause of burn infections and colonizer medical devices like catheters [2]. *P.aeroginosa* is rapidly resistant to an extensive range of antibiotics. They are also an opportunistic pathogen of plants and secrets a variety of pigments, like pyocyanin, pyoverdin and pyorubin [3].

Staphylococcus aureus is a gram-positive, facultative anaerobic [1], grape shape that can found as a part of the normal skin flora on the skin and nasal passages. It can cause of many type of skin diseases such as pimples, cellulites folliculitis, impetigo, abscesses, burn infections and scalded skin syndrome, also it can cause of dangerous illnesses like pneumonia, meningitis, osteomyelist endocarditis, toxic shock syndrome (TSS), sepsis and bacteremia [4].

M. Shafighi is with Young Researchers Club, Falavarjan Branch, Islamic Azad University- Isfahan- Iran. (Corresponding author to provide phone: +989133051023; fax: +98-0312-3120136; e-mail: asham987@gmail.com).

Z. Emami is with Department of Microbiology, Falavarjan Branch, Islamic Azad University- Isfahan- Iran. (e-mail: zarrindokht4@gmail.com).

M. Shahsanaei is with Young Researchers Club, Falavarjan Branch, Islamic Azad University- Isfahan- Iran.

E. Khaliliyan is with Young Researchers Club, Falavarjan Branch, Islamic Azad University- Esfahan- Iran.

The duration of treatment depends on severity and the organ of infection. The common antibiotic choice for *S. aureus* is penicillin, but unfortunately the penicillin resistance has been increased.

The life science society is primarily concerned with the infectious and pathogenic bacterial diseases.

Probiotic strains with many types of applications are the best alternative treatment for the variety of infectious diseases [5].

Probiotic lactobacilli have many beneficial properties to control pathogenic microorganisms. These include the ability to regulate gut microbiota, producing antimicrobial substances, stimulating protective immune response, prevention faecal enzymatic activity, producing short chain fatty acids allowing an advisable acidification of the gut [6].

Lactic Acid Bacteria (LAB) are usually aero tolerant fermentative microorganisms. The inhibitory properties of LAB is due to the accumulation of main primary metabolites includes lactic and acetic acids, ethanol and carbon dioxide as well as other antimicrobial compounds such as formic and benzoic acids, hydrogen peroxide, diacetyl, acetoin and bacteriocins[7].

LAB has been isolated from several foods, including dairy products, meat products, plants, sewage, manure animals and also humans [8].

Table olive is one of the plants, which have Probiotic properties. There is several studied with the aim of determination of valuable compounds in olives, but a few studies have been shown their probiotic properties [9, 10].

In this study, we isolated several strains of lactic acid bacteria from Iranian native olives and tested their antibacterial activities against *P.aeroginosa* and *S.aureus.*

II. Materials and Methods

A. Olive Processing

Three type of olive includes snaky olive, yellow olive and oily olive collected from Tarom-Iran, in September 2010. Each type of olives divided in 6 groups, which were suspended in 5% NaCl (Merck, Darmstadt, Germany) solution, 10% NaCl solution, 5% NaCl and 1% Sodium hydroxide (Merck, Darmstadt, Germany) solution, 10% NaCl and 1% Sodium hydroxide, 5% NaCl and 3% Sodium hydroxide solution, 10% NaCl and 3% Sodium hydroxide solution, respectively [6, 11].

B. Isolation of labs

Lactic Acid Bacteria were isolated from the brine of each sample in the prior of time. If it was necessary, brine was diluted. The samples were spread on Man-Rogosa-Sharpe (MRS) agar (Merck, Darmstadt, Germany) for isolation of lactobacillus and M17 (Merck, Darmstadt, Germany) for lactococcus. They were incubated for 72 hours at $30°_c$. For initial identification, each colony was tested for gram staining and catalase test. The gram positive, catalase negative rod shape bacteria was determined as *Lactobacillus sp.*, that shown by LB_1, LB_2, LB_4, LB_6, LB_7, LB_8, LB_{10}, LB_{11}, LB_{12}, LB_{13}, LB_{14}, LB_{1*}, LB_{2*}, LB_{3*}, LB_{4*}, LB_{5*}, LB_{6*}, LB_{7*}, LB_{9*}, LB_{10*}, LB_{11*}, LB_{13*} and LB_{14*}. The gram positive, catalase negative coccid was determined as *Lactococcus sp.*, which shown by LC_3, LC_5, LC_9, LC_{8*} and LC_{12*}.

C. Pathogenic Bacteria

P.aeroginosa PTCC 1074 and *S.aureus* PTCC 1112 were obtained from Persian type culture collection. The lyophilized strains were activated in Muller Hinton Agar (MHA) (Merck, Darmstadt, Germany) and subculture at $37°_c$ in MHA before each experiment.

28 strains of labs that isolated from olives were maintained at $4°_c$ on MRS agar, then subculture at $37°_c$ in MRS broth for 24_h before each experiment to increase the number of the labs [8]. The labs were separated in ependorf tubes and centrifuged (Centrifuge 5415r Refrigerated Ependorf) at $10°_c$ in 313g for 5 minutes. The supernatant was strewed and pellet was used to inoculation in wells or at blank disks.

D. Antimicrobial Activities

P.aeroginosa and *S.aureus* were suspended in Tripton Soya Broth (TSB) (Merck, Darmstadt, Germany). The number of pathogenic bacteria was stimulated by comparison to McFarland Standard 0.5. 100µl of the suspension was spread on to the MHA plates.

The antimicrobial activity of isolated labs was determined by two methods. In disk diffusion assay, 20µl of each pellet was inoculated to blank disks.

In agar well diffusion, 40µl of each pellet was inoculated to each well.

Diameters (in mm) of growth inhibition zones were measured after incubation at $37°_c$ for 24_h.

Chloramphenicol disk and Gentamicin disk were used as a positive control for *S.aureus* and *P.aeroginosa* respectively. MRS broth was used as a negative control.

III. Results

A. Strains

28 strains of LAB that several of them have antibacterial activity were isolated from 3types of Iranian native olives.

B. Antimicrobial Activities

After 24_h incubation at $37°_c$, inhibition zones diameters were measured; Antibacterial effect of labs by disk diffusion assay method is shown in Table I and Figure 1. A strong inhibition zone of *P.aeroginosa* and *S.aureus* were obtained by LB_{9*}, 14 mm, and by LC_{8*}, LC_{12*}, LB_{14*}, 10 mm respectively.

P.aeroginosa and *S.aureus* are resistant to LC_3, LB_4, LC_5, LB6, LC_9, LB_{13}, LB_{14}, LB_{11*}, LB_{13*}, LB_{14*}, and to LB_4, LC_9, LB_{11}, LB_{13}, LB_{14}, LB_{1*}, LB_{2*}, LB_{3*}, LB_{6*}, LB_{7*}, LB_{10*}, LB_{11*}, LB_{13*}, respectively.

TABLE I
ZONE OF INHIBITION (ZOI) IN DISK DIFFUSION ASSAY METHOD

Strain's number	ZOI (mm)	
	P.aeroginosa	*S.aureus*
1	9	9
2	11	9
3	R	9
4	R	R
5	R	9
6	R	8
7	7	9
8	8	7
9	R	R
10	10	8
11	7	R
12	7	9
13	R	R
14	R	R
1*	7	R
2*	9	R
3*	9	R
4*	10	9
5*	9	9
6*	12	R
7*	10	R
8*	11	10
9*	14	9
10*	9	R
11*	R	R
12*	12	10
13*	R	R
14*	R	10
+	15	20
–	R	R

(R): Resistance

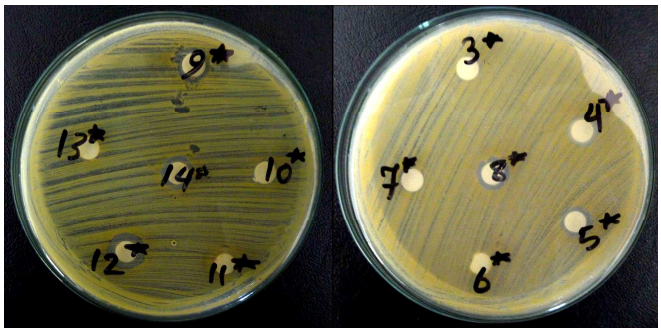

Fig. 1 The inhibitory effect of labs by disk diffusion assay method against *S.aureus.*

Antibacterial effect of labs by agar well diffusion method is shown in Table II and Figure 2. The maximum inhibition zone of *P.aeroginosa* and *S.aureus* were obtained by LB_1, 13 mm, and by LB_7, 13 mm respectively.

P.aeroginosa and *S.aureus* are resistant to LC_3, LB_4, LC_5, LB_6, LC_9, LB_{13}, LB_{14}, LB_{6*}, LB_{10*}, LB_{11*}, LC_{12*}, LB_{13*}, LB_{14*}, and to LB_4, LC_9, LB_{11}, LB_{13}, LB_{14}, LB_{1*}, LB_{2*}, LB_{6*}, LB_{11*}, LB_{13*} and LB_{14*} respectively.

TABLE II
ZONE OF INHIBITION (ZOI) IN AGAR WELL DIFFUSION METHOD

Strain's number	ZOI (mm)	
	P.aeroginosa	*S.aureus*
1	13	9
2	12	8
3	R	8
4	R	R
5	R	11
6	R	10
7	11	13
8	7	9
9	R	R
10	8	10
11	8	R
12	11	8
13	R	R
14	R	R
1*	7	R
2*	8	R
3*	8	10
4*	1	12
5*	1	8
6*	R	R
7*	8	12
8*	6	7
9*	8	9
10*	R	8
11*	R	R
12*	R	7
13*	R	R
14*	R	R
+	15	20
–	R	R

(R): Resistance

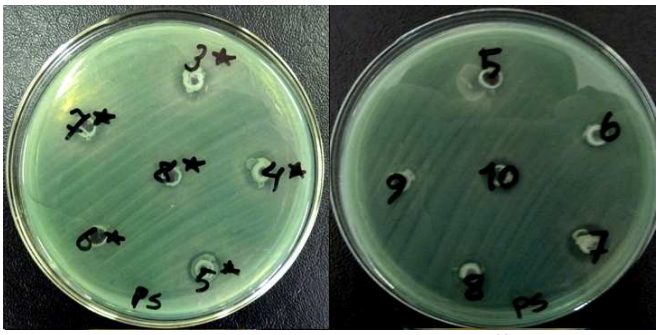

Fig. 2 The inhibitory effect of labs by agar well diffusion method against *P.aeroginosa*

IV. Discussion

The aim of this study was to determination of antibacterial characteristics of Lactic Acid Bacteria isolated from naturally fermented Iranian native olives against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

The result of disk and well diffusion agar against these pathogens were confirmed each other. The size of inhibition zone was different according to the type of bacteria, the method and the concentrations of labs [5].

Using different methods of de-bitter had not any statistically significant effect on the number of labs were isolated.

S.aureus was resistance to the samples LB_{3*}, LB_{7*} and LB_{10*} in disk diffusion assay. It was due to the less dose of inoculation on disk in comparison of wells.

Based on the results obtained from disk and agar well diffusion methods, it can be suggested that in comparison with gram-positive bacteria, the growth of gram-negative bacteria is inhibited at higher concentrations of labs. Yoda et al. (2004) have reported the same results, emphasizing on the higher susceptibility of gram-positive bacteria in comparison with gram –negative bacteria which could be related to differences in cell wall structure, cell physiology, metabolism or degree of contact [12]. But Ping Su et al. (2008) had reported that the antimicrobial activity of labs did not have significant relationship with the gram reaction of bacteria [13].

The samples LB_4, LC_9, LB_{13}, LB_{14}, LB_{6*}, LB_{11*}, LB_{13*} were not shown any antibacterial activity against *P.aeroginosa* and *S.aureus*.

Acknowledgment

This work was supported by Falavarjan Branch, Islamic Azad University. The authors also thank Dr. Ramesh Monajjemi for her aid.

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