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PROBIOTIC POTENTIAL OF SOME *LACTOBACILLUS* STRAINS ISOLATED FROM RAW MILK

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ABSTRACT

A total of 25 samples of raw cow milk, were collected randomly from street vendors and supermarkets in Zagazig city, Egypt for detection of the probiotic potential of some isolated *Lactobacillus* strains from raw milk. The obtained results revealed that the *Lactobacillus* Strains were detected in all the examined samples. The isolated strains were slightly affected by pH 3 and bile condition (0.5%). The obtained results displayed resistance against Erythromycin, Gentamycin and Tetracycline. Meanwhile, Ampicillin, Chloromphnicol, Steptomycin and Vancomycin exhibited an effect against some strains. The isolated strains produced γ-hemolysis, NaCl tolerance was positive for these strains. Also, β galactosidase was produced.

Keywords: Probiotic Potential, *Lactobacillus* Strains, Raw milk

INTRODUCTION

Probiotics are non-pathogenic microorganisms, which on ingested in adequate amount exert a positive health benefit on host (FAO/WHO, 2006). The search for more new probiotics is driven by the growing demand for probiotic functional food and beverages and dietary supplements due to rising levels of health consciousness and growing consumer awareness regarding gut health and the concept of preventive health care. It is now well established that some of the infections and disorders in the human body, such as irritable bowel syndrome, inflammatory bowel disease, and antibiotic-induced diarrhea, could be due to deficient or compromised intestinal microflora, and probiotics have been considered to be one of the disease control strategies to overcome such disorders (Dunne *et al.,* 2001).

Lactic acid bacteria (LAB) are most commonly studied probiotic for the past few decades. These are desirable microflora of thegastro intestinal tract (GIT) and are thus 'generally regarded as safe' (Tannock, 1997). Secondly, they are involved in the fermentation and are dominant microflora of fermented products. They are known to play an essential role in food preservation and inhibit spoilage microorganisms or food borne pathogens by production of lactic acid, acetic acid, H_2O_2, bacteriocin, diacetyl and CO_2 (Nur and Aslim, 2010).

Probiotic bacteria produce antimicrobial compounds whichmay exhibit either bactericidal or bacteriostatic properties (Pringsulaka *et al.*, 2015). The probiotic microorganisms mainly consist of the strains of the genera *Lactobacillus* and *Bifidobacterium*,*Streptococcus* and some *Enterococcus* species (Morrow *et al.*, 2012).

The ability to survive and grow in low pH environment is characteristic for probiotics (Marteau *et al.*, 1997). Bile tolerance of microorganisms has been used as a selective criterion for potential probiotics (De Smet *et al.*, 1995). Bile resistance of some strains is related to specific enzyme activity-bile salt hydrolase (BSH) which helps hydrolyze conjugated bile, thus reducing its toxic effect (Du Toit *et al.*, 1998).

An important property of the probiotic strains is their antagonistic activity against pathogenic bacteria either by competitive exclusion, decrease of redox potential, interbacterial aggregation, or production of antimicrobial substances including organic acids, other inhibitory primary metabolites such as hydrogen peroxide, and special compounds like bacteriocins and antibiotics (Kalantzopoulos, 1997).

Numerous clinical studies demonstrate that organisms of the *Lactobacillus* genus are both preventative and therapeutic in controlling intestinal infections when administered with milk containing these organisms (Gilliland, 1990).

Antibiotic resistance of probiotic strains assures maintenance of healthy intestinal microbiota throughout antibiotic treatments of microbial infections. LAB display a wide range of antibiotic susceptibilities and resistances. In most cases, antibiotic resistance is not transmissible, but represents an intrinsic characteristic of the organism. An important requirement for probiotic strains is that they do not harbor mobile elements carrying resistance genes (Salminen *et al.*, 1998).

The objectives of this study were to characterize some *Lactobacillus* species, isolated from Egyptian raw milk according to the requirements for probiotics in order to consider their further application in the development of

new functional products.

MATERIALS AND METHODS

Collection of samples

A total of 25 samples of raw cow milk, were collected randomly from street vendors and supermarkets in Zagazig city, Egypt. All samples were aseptically collected in sterile containers and transported rapidly in a 4 °C vehicle mounted refrigerator to laboratory of Food Control, Faculty of Veterinary Medicine, Zagazig University, Egypt to be investigated microbiologically within few hours.1 ml of sample was taken in 9 ml of MRS Broth (Hi-Media, India) and incubated at 37°C for 48 h. One loopful broth culture was streaked on MRS agar plates and incubated 48 hrs. Suspected single colonies were isolated and identified by gram staining and short biochemical tests (MacFaddin, 2000; Bergey *et al.*, 1994). Single colony was stored in MRS agar slant for further study.

Gram staining

Gram staining test was performed for all isolated strains according to the standard procedure. A smear of single colony was prepared on a clean glass slide and the smear was allowed to air-dry and then heat fixed. The heat fixed smear was flooded with crystal violet solution and after one minute, it was washed with water and flooded with mordant Gram's iodine. The smear was decolorized with 95% ethyl alcohol and rinsed with water. Finally, safranin was used as counter stains for 60-80 sec and washed with water, and examined under oil immersion (100X). *Staphylococcus aureus* ATCC 25923 and *Escherichia* coli ATCC 25922 were used as positive and negative control, respectively.

Catalase test

A drop of 3% hydrogen peroxide was added to a fresh culture on a sterile glass slide and mixed well. Producing bubble or froth, indicated catalase-positive and no bubble or froth indicated catalase negative. Staphylococcus aureus ATCC 25923 and E. coli ATCC 25922 were used as positive and negative control, respectively.

Kliger's Iron Agar (KIA) test

All isolates were tested for KIA test to know the mode of glucose and lactose utilization. Fresh culture was inoculated by stabbing the butt and streaking the slant. After incubation at 37°C for 24 h, results were recorded for color changes of the butt or slant, H_2S or other gas production. The results were observed as alkaline slant and acid butt for fermentation of glucose only, acid slant and alkaline butt for fermentation of lactose only, acid in both slant and butt for fermentation of both lactose and glucose whereas alkaline in both slant and butt for fermentation of neither lactose nor glucose. Production of hydrogen sulphide made blacking of the medium and the gas production give rise to bubble formation in the tube. S. aureus ATCC 25923 was used as positive control.

Evaluation of probiotic properties

Acid and bile tolerance

The experiment for tolerance of isolate to pH 3.0 and 7.0(control) was performed following the method described by Yu *et al.,* (2013). The ability of the isolates to grow in the presence of 0.5% of bile (w/v) was determined according to the method of Vinderola and Reinheimer (2003). Resistancewas evaluated by plate count onMRS agar.

Antibiotic resistance

MRS agar plate was overlaid with 100 ml of bacterial culture containing 10^8CFU/ml and antibiotic discs ofAmpicillin, Chloramphenicol, Erythromycin, Gentamycin, Streptomycin, Tetracycline, and Vancomycin were placed on inoculated plates under sterile conditions. Afterincubation for 24 h at 30 °C, the diameter (mm) of inhibition zonewas measured.

Antibacterial activity

Agar well diffusion method was used to test the antimicrobialactivity as described by Mishra and Prasad (2005). The supernatants of 18-20 h grown *Lactobacillus* cells were tested against *E.coli* and *S. aureus*.

b-Galactosidase activity

For b-galactosidase activity, bacterial cultures were streaked onMRS agar plates containing 60 ml X-gal (5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside) and 10 ml of IPTG (iso-propyl-thio-b-D galactopyranoside) solution as inducer.

Haemolytic activity

All the *Lactobacillus* isolates studied for haemolytic activity gave negative result.

Salt tolerance assay

The salt stress response of the selected isolates (10^9CFU/ml) were screened in MRS broth containing differentconcentrations of NaCl ranging from 0 to 10% (w/v) asdescribed by Adnan and Tan (2007).

RESULTS AND DISCUSSION

The results showed in Table (1) exhibited that *Lactobacillus acidophilus* and *Lactobacillus casei* were the most common isolated strains; while, *Lactobacillus brevis* and

Lactobacillus fermentum were the less prevalent strains. Similar results were obtained by Aziz *et al*., (2009) which detected *Lactobacillus acidophilus* in 25% of the examined raw milk in Pakistan. On contrarily, Asuman and Emin (2018) isolated lower incidence (1.79%) of *Lactobacillus acidophilus* in cow milk. Meanwhile, Alnakip *et al.,* (2016) detected lower incidence (2.44%) of *Lactobacillus casei* in the examined cow raw milk in Sharkia Governorate, Egypt.

Table (1): Incidence of isolated *Lactobacillus* strainsfrom raw milk.

Lactobacillus sp. Isolates	No	%
Lactobacillus plantarum	8.00	14.55
Lactobacillus acidophilus	14.00	25.45
Lactobacillus casei	12.00	21.82
Lactobacillus brevis	5.00	9.09
Lactobacillus rhamnosus	9.00	16.36
Lactobacillus fermentum	7.00	12.73
Total	55.00	100.00

Table (2): Resistance of selected *Lactobacillus* strains towards acidic conditions (PH 3 and 7).

Strain	Count (Log)	
	pH7	pH3
Lactobacillus plantarum	8.81±0.01	8.72±0.20
Lactobacillus brevies	9.25±0.05	8.69±0.01
Lactobacillus rhamnosus	9±0.01	8.83±0.18

Each value in the table represents the mean value ± standard deviation (SD) from triplicate.

Table (2) revealed relative resistant of different strains of *lactobacillus* against pH7 and pH 3. Nearly similar results were obtained by Dowarah *et al*., (2018).

Table (3): Resistance of identified selected *Lactobacillus* strains to bile condition (0.5%).

Strain	Count (Log)	
	Control	0.5%
Lactobacillus plantarum	9.55±0.04	9.31±0.06
Lactobacillus brevies	9.22±0.09	8.88±0.04
Lactobacillus rhamnosus	9.46±0.18	9.02±0.19

Each value in the table represents the mean value ± standard deviation (SD) from triplicate.

Concerning resistant of *lactobacillus* strains to bile condition (0.5%), Table (3) showednon-significant reduction of the bacterial count. These results agreed with those obtained by Angmo *et al.*, (2016) and Halder *et al,* (2017).

Table (4) Antibiotic sensitivity test of identified selected *Lactobacillus* strains

LAB Strains	AMP	C	E	CN	S	TE	VA
Lactobacillus plantarum	R	S	R	R	S	R	S
Lactobacillus brevies	R	R	R	R	R	R	R
Lactobacillus rhamnosus	S	R	R	R	R	R	R

R: resistance, S: susceptible, AMP: Ampicillin, C: Chloramphenicol, E: Erythromycin, CN: Gentamycin, S: Streptomycin, TE: Tetracyclin, VA: Vancomycin.

Table (4) showed the antibiotic sensitivity of the different *lactobacillus* strains. The obtained results displayed resistance against Erythromycin, Gentamycin and Tetracyclin. Meanwhile, Ampicillin, Chloromphnicol, Steptomycin and Vancomycin exhibited an effect against some strains. These results coincided with those detected by Wang *et al*., (2019) which obtained a sensitivity of some *lactobacillus* strains to Gentamycin, Steptomycin and Vancomycin. Moreover, Sirichoat *et al.*, (2020) showed sensitivity of *lactobacillus* strains to Steptomycin; while, these strains were resistant against Gentamycin, Vancomycin, Ampicillin and Tetracyclin.

Table (5) Blood haemolysis of identified selected *Lactobacillus* strains

LAB strains	Blood haemolysis
Lactobacillus plantarum	γ-hemolysis
Lactobacillus brevies	γ-hemolysis
Lactobacillus rhamnosus	γ-hemolysis

The obtained results showed in Table (5) revealed hemolytic effects of *lactobacillus* strains (γ-hemolysis), similar results were detectedHalder *et al.,* (2017).

Table (6): NaCl tolerance of identified selected *Lactobacillus* strains.

LAB strains	NaCL 4% tolerance
Lactobacillus plantarum	+
Lactobacillus brevies	+
Lactobacillus rhamnosus	+

Regarding NaCl tolerance, Table (6) showed positive tolerance of *lactobacillus*strains to sodium chloride solution (4%). Nearly same findings recorded Ragul *et al.*, (2017).

Table (7): β- Galactosidase production by identified

selected *Lactobacillus* strains

LAB strains	β-Galactosidase production intensity
Lactobacillus plantarum	++
Lactobacillus brevies	+
Lactobacillus rhamnosus	++

-: No activity, +: Weak activity, ++: Strong activity.

β galactosidase, commonly known as lactase, represents commercially important enzyme that is prevalently used for lactose hydrolysis in milk and whey. To the date, it has been isolated from various sources. The obtained results in Table (7) revealed positive β-Galactosidase production intensity with different strains of *lactobacillus*. Carević *et al.,* (2015) detectedβ galactosidase production by *L. acidophilus*, *L. reuteri, L. helveticus* and *L. delbrueckii* sub sp. *bulgaricus.* Meanwile*, L. rhamnosus* could not produce β galactosidase.

Table (8) Inhibition zones (mm) of *Lactobacillus* strains showing antibacterial activity against *S. aureus* and *E. coli* after neutralization.

Isolates	Diameter of inhibition zone (mm)	
	S.aureus	*E. coli*
Lactobacillus plantarum	7	14
Lactobacillus brevies	9	13
Lactobacillus rhamnosus	3	9

Concerning the inhibition effects of *Lactobacillus* strains against both *S.aureus* and *E. coli*; Table (8) displayed clear inhibition zones of *Lactobacillus plantarum* and *Lactobacillus brevies* against the bacterial cultures. However, the inhibitory effects of *Lactobacillus rhamnosus* strain against the *S. aureus* was found to be insignificant. Previous studies recorded the inhibitory effects of *lactobacillus* strains in opposition to *E. coli* (Davoodabadi *et al.*, 2015 and Poppi *et al.*, 2015). Moreover, Melo *et al.*, (2016) obtained the inhibitory effects of *lactobacillus* strains against *S.aureus*.

From the above-mentioned results, it was concluded that the identified *Lactobacillus* strains have probiotic potential. However, further investigations and safety testing should be applied.

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