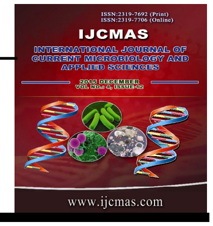
Int.J.Curr.Microbiol.App.Sci (2015) 4(12): 548-554



International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 12 (2015) pp. 548-554**
http://www.ijcmas.com

Original Research Article

Analysis of Probiotic properties of *Lactobacillus acidophilus* from commercial yoghurt

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Keywords

Lactobacillus acidophilus, Commercial yoghurt, Antimicrobial and Nacl

A B S T R A C T

Lactic acid bacteria are very significant to human health due to the production of some antimicrobial substances and ability to inhibit pathogenic bacteria. Furthermore, the bacteria are also used as starter culture in the production of various foods. The present study was focused on isolation and characterization of Lactobacillus acidophilus from yogurt and to demonstrate some of probiotic properties of these isolates. All isolates were phenotypically characterized including studying, biochemical, effect of sodium chloride and pH during growth, carbohydrates test and characterizing the antimicrobial activity of *Lactobacillus acidophilus* against pathogens. The present study demonstrates that *Lactobacillus acidophilus* produced a bacteriocin- like inhibitory substance with a broad spectrum of antimicrobial activity directed against pathogenic indicator organism suggesting its protective value against enteric pathogens.

Introduction

Certain species of Lactobacilli are important and are gaining increasing attention in food fermentation industry because of their biotechnologically interesting properties (Roy et al .,2000).Based on their “Generally regarded as safe”(GRAS) status, Lactobacilli have been extensively studied for their molecular biology in order to improve their specific beneficial characteristics (Pouwels, and Leerm, 1993). The largest group of probiotic bacteria in the intestine is lactic acid bacteria (LAB). Probiotics are live microorganisms that are similar to beneficial microorganisms found in the human gut, and have emerged as a major balancing factor influencing gastrointestinal physiology and function (Diplock et al.,2001).In food industry, LAB are widely used as starter cultures and have been recognized to be part of human microbiota (Holzapfel et al., 2001).In raw milk and dairy products such as cheeses, yoghurts and fermented milks, Lactobacilli are naturally present or added intentionally, for technological reasons or to generate a health benefit for the consumer, and therefore yoghurt is one of the best-known foods that contain probiotics (Oskar et al., 2000). The genus Lactobacillus consists of a genetically and physiologically diverse groups of Gram-positive, rod shaped, catalase negative and non-spore forming bacteria (MacFaddin,2000). Certain Lactobacillus strains are considered important owing to their role in various foods and feed fermentations, production of many important metabolites and owing to their role in the prevention of food spoilage. Furthermore, they play a role in combating intoxication and infection by acting as antagonists against other pathogens through the production of antimicrobials and bacteriocin (Holzapfel et al., 2001), (Hirano et al., 2003). The aim of this study was to demonstrate some of probiotic properties of these isolates.

Materials and Methods

This study has been conducted between September 2014 to January 2015, at the laboratories of the Biology Department, Babylon University.

Collection of Samples

Sixty samples of commercial yoghurt were collected from local markets of Babylon province of Iraq. Immediately after collection, the samples were stored in sterile containers at 4°C.

Media and Samples Preparation

Growth media used in this study were MRS broth (Himedia, India), MRS agar (Himedia, India), nutrient agar (Oxoid, England). Media were prepared according to manufacturer's instructions. All media and instruments were autoclaved for 15 min at 121°C before use. One gm. of each sample was separately suspended in 100 ml of MRS broth of pH 6.5 and homogenized. A fivefold dilutions were made from each homogenized sample and all dilutions were incubated for 24 hours at 37°C under anaerobic condition in the presence of 5 % CO2. A loopful of each culture was streaked on to the MRS agar plate and plates were incubated under anaerobic condition at 37°C for 24 hours (Sneath et al., 2009). Finally, a single colony of *Lactobacillus* was isolated based on their colony morphology and specific biochemical tests (Gram staining, oxidase, catalase, motility test, starch hydrolysis, growth at 10°C, 15°C and 45°C in MRS broth, growth in (2, 4, 6, 8, 10 and 12) percent NaCl as a tolerance test. Fermentation of carbohydrates were determined as described by [8] including glucose, L-xylose, D-fructose, Mannitol, Raffinose,D- mannonse,Ribose and lactose.

Determination of Optimal Growth

For the determination of pH for optimum growth of the isolates, 100µl overnight culture of the isolates was inoculated into MRS broth with varying pH ranging from (3.5- 9.5). The pH was adjusted with concentrated Hcl or NaOH .The inoculated broths were incubated under anaerobic condition for 24 h at 37°C in the presence of 5% CO2. Bacteria growth was measured using a spectrophotometer at 560 nm (Hoque et al,.2010).

Measurement of NaCl Tolerance

For the determination of Nacl tolerance, all isolates were grown in MRS broth supplemented with different concentrations of Nacl (2,4, 6, 8, 10 and 12 percent) that were inoculated after sterilization with 1% (v/v) of overnight culture of *Lactobacillus* and then were incubated an aerobically for 24h at 37°C.The bacterial densities were determined by visual measurement of their turbidity and were classified as Maximum growth (++), normal growth (+), and no growth (-) (Hoque et al,.2010).

Antimicrobial Activity against Indicator Organisms

Antimicrobial effects of *Lactobacillus acidophilus* against (*E.coli, Klebsiella pneumoniae, Enterococcus faecalius, Aeromonas hydrophila, Pseudomonase aeruginosa, Proteus mirabilis* and *Salmonella typhi*) were determined by the agar diffusion method. Overnight cultures of the indicator strains were used to inoculate agar growth media (BHI Himedia, India) plates that were incubated at 37°C. Wells of 5mm diameter were cut into the plates. To detect antibacterial activity of *Lactobacillus acidophilus* 10 ml of broth was inoculated with each strain of *Lactobacillus acidophilus*., and was incubated at 37°C for 48 h, cell free – solution was obtained by centrifuging the culture (6000 x g for 15 min),and then were followed by filtration of the supernatant through a 0.2 μm pore size, the filtered supernatant were neutralized by 1N NaOH to pH 6.5, then 50μl of supernatant fluid was added to each well and incubated at 37°Cfor 24 h followed by measurement of growth inhibition zones (Topisirovic et al., 2006).

Results and Discussion

Isolation and Identification

Seven Lactobacillus-suspect bacterial isolates were obtained from 60 samples of yoghurt .The isolates were Gram positive, rod-shaped, oxidase negative, catalase negative, non-motile, indole- negative and starch hydrolysis negative. Their characteristics are listed in table 1.The carbohydrates fermentation patterns of the isolates are listed in Table 1.

pH and Optimal Growth

The influence of pH was tested in a range of highly acidic (pH 3.5) to neutral (pH 7.5) and alkaline (pH 9.5). We have observed the maximum growth, by measurement of bacterial densities of L7 to be at pH 5.5 . The results of experiment are shown in Fig (1). There is a strong correlation between the pH and the growth of the Lactobacilli, the maximum growth was enhanced when the culture was controlled at pH 5.5 .Survival could also be observed that at acidic pH value of 3.5.

Influence of Various Nacl Concentrations

Isolated Lactobacilli were able to tolerate growth 2, 4, 6, 8, 10 and 12 percent of Nacl in MRS broth.. However, bacterial growth was correlated with various Nacl concentrations in the media with optimal growth being optimal at 4 percent Nacl while concentrations of 10% and 12% Nacl significantly inhibited the growth of Lactobacilli with exception of L1 and L7 that could grow at this Nacl concentration. Results of the experiment are shown in Table 2.

Antimicrobial Activity

Isolates of pure local cultures for *E.coli*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Salmonella typhi* were kindly provided by Hilla hospital microbiology lab. Babylon, Iraq, and their identifications were confirmed according to (Holt et al., 1994). Antibacterial activity of cell – free supernatant was evaluated on the provided clinical strains of *E.coli*, *Klebsiella pneumonia*, *Enterococcus faecalius*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Salmonella typhi* using agar well diffusion method (Topisirovic et al., 2006).

Table.1 Biochemical Test Results of *Lactobacillus acidophilus*

Morphological & biochemical	L1	L 2	L 3	L 4	L 5	L 6	L 7
Gram stain	+	+	+	+	+	+	+
Oxidase test	-	-	-	-	-	-	-
Catalase test	-	-	-	-	-	-	-
Motility test	-	-	-	-	-	-	-
Starch hydrolysis	+	-	+	-	+	-	-
Indol test	-	-	-	-	-	-	-
Growth at 10 ˚C	+	-	-	-	±	±	-
Growth at 15 ˚C	-	-	-	-	-	-	-
Growth at 45 ˚C	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+
Ribose	-	-	-	-	-	-	-
D- fructose	+	+	+	+	+	+	+
L- xylose	_	_	_	_	_	_	_
Mannitol	-	-	-	-	-	-	-
D- mannonse	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+

Table.2 Tolerance to Nacl Results of *Lactobacillus acidophilus*

Con..of Nacl No. % of isolates	2%	4%	6%	8%	10%	12%
L1	+	++	++	+	+	+
L 2	++	+	+	+	_	_
L 3	+	++	+	+	_	_
L 4	+	++	+	+	_	_
L 5	+	++	+	+	_	_
L6	++	++	+	+	_	_
L 7	++	++	+	+	+	+

Figure.1 Optimal pH of Isolated *Lactobacillus Acidophilus*

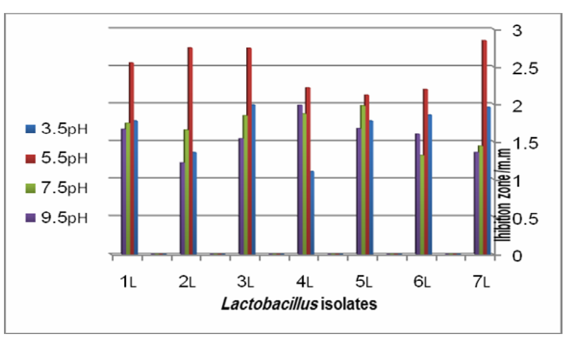
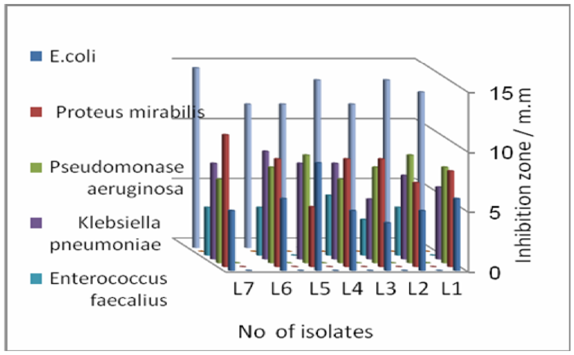


Figure.2 Antimicrobial Activity of *Lactobacillus acidophilus* against 7 Indicator Bacterial *spp*



The results of the experiment showed that the *Aeromonas hydrophila* is the most affected by filtered supernatant of the seven *Lactobacillus acidophilus* among the clinical isolates while the *Salmonella typhi* and *E. faecalis* were least affected, whereas the rest of the isolates showed varying response towards the filtered supernatant, Fig(2). The results of present study, showed that L7 have a broad spectrum of activity against the tested clinical bacterial isolates.

In the present study, all physiological and biochemical characteristics of the Lactobacilli isolated from the dairy products were identical to those reported by (Cullimore,2000). Characterization of ten selected isolates was initially conducted by cell morphology and by physiological and biochemical tests. Results of these tests and their limits are shown in Table 1. The majority of the LAB possesses an inducible acid tolerance response (ATR) which is also known as the acid adaptive response. This property improves the survival of adapted cells upon exposure to lethal acid challenge (Elizeter, and Carlos,2005). pH is an important factor which can dramatically affect bacterial growth. In the present study, we assessed the growth of *Lactobacillis acidophilus* in various ranges of pH (3.5-9.5), to determine the pH for optimum growth. In this study, it was found that the L7, had a maximum growth (OD =2.850) at pH 5.5 that exceeded the growth of other Lactobacilli which had their optimal growth at different pH, Fig (1). Nacl is an inhibitory substance which may inhibit growth of certain types of bacteria. Growth at different Nacl concentrations was observed; all of the isolates have the ability to grow at 2, 4, 6, and 8 percent concentrations of Nacl. Growth of all isolates was inhibited at (Topisirovic et al., 2006 and Cullimore,2000) % Nacl concentration, however L1 and L7 could grow at this concentration. Our experimental results are in agreement with the findings of (Cullimore,2000), in case of Lactobacilli isolated from gastrointestinal tract of swine that were tolerable to 4-8 % Nacl. The growth-inhibiting activity of LAB to other bacterial spp has generally been attributed to the fact that *Lactobacillus spp* lower the pH and / or produce lactic acid, for example strains of *L. acidophilus, L. casei sub sp, rhammnosus and L. bulgaricus* inhibited the growth of clinical isolates of *H.pylori* (Midolo et al., 1995), while L. caseisubsp rhammnosus strain Lcr 35 reduced the growth of enteropathogenic *E. coli* and *Klebsiella pneumonia* (Forestier et al., 2001). The data reported by Fayol-Messaoudi et al.,(2005) showed that the strains induce complete inhibition of the growth *Salmonella spp* that results mainly from the effect of an acidic pH .Antimicrobial activity of Lactobacillus strains against bacterial pathogens was revealed to be multifactorial and to include the production of hydrogen peroxide , lactic acid, bacteriocin-like molecules and unknown heat – stable , non-lactic acid molecules (Servin,2004; Olanrewaju,2007) .

In conclusion, results of this study suggest that only a few Lactobacillus isolate may possess unique characteristics that worth's further investigation for the identification of the mechanism of it antimicrobial effect against specific pathogens as well as its ability for optimal growth and survival in the presence of high concentrations of Nacl in their growth medium. We conclude that Lactic acid bacteria (LAB) from yogurt may act as are servoir of antimicrobial-resistance genes. These bacteria could act as bio-therapeutic microorganisms and might be good candidates to overcome the growing challenge of nosocomial infections due to multi-drug resistant strains.

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