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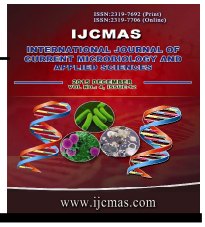

Original Research Article

Probiotic Properties of Some Lactic Acid Bacteria Isolated from Egyptian Dairy Products

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Keywords

Lactic acid bacteria, Probiotic properties, Dairy products, β-haemolysis, Bile salts

A B S T R A C T

The aim of this study was planned to evaluate probiotic properties of some lactic acid bacteria (LAB) previously isolated from fermented dairy products. These microorganisms had good functions potentially applicable in the dairy and food industries. To a chive this purpose eight strains as follows: *hamnosus* NRC AM-6, *Lactobacillus plantarum* NRC AM-7, *Lactobacillus pentosus* NRC AM-(5, 8), *Pediococcus pentosaceus* NRC AM-(1, 4), *Lactobacillus brevis* NRC AM2 (1 strain) and *Lactoccocus lactis* ssp. *Lactis* NRC AM-3 were screened in vitro for their probiotic traits, tolerance to bile salts, low pH resistance, growth and viability in different concentrations of NaCl and phenotypic safety assessment. The results indicated that survival (log cfu/ ml) of all tested strains were affected and gradually decreased from 4 to 5 log cycles with increasing the bile salts concentration to 1 % meanwhile, a good survival was observed with all tested strains under acidic conditions (pH 2) till 5 hrs of incubation time at 37^{o}C but there was not any growth after 24 h. Also, no haemolytic activities (β-haemolysis) were observed for all tested strains.

Introduction

Probiotics have become a major focus of lactic acid bacteria research over the past 10 years with most attention drawn to the genera *Lactobacillus* and *Bifidobacterium* for improving human health in natural way (Fernandez *et al*., 2003). These organisms have been widely reported to exert many beneficial health effects, such as activation of the immune system, prevention of cancer cell growth, maintenance of mucosal integrity and presentation of an antagonistic environment for pathogens (Rashmi and Gayathri, 2014). Researchers who work in the food industry and research centers pay more attention to the identification of new probiotic bacteria with better performance characteristics as well as investigation of their performance because these findings can be very effective in promoting sale and consumption of these products (Niazi Amraii *et al*., 2014). Probiotics are defined by the FAO/WHO as "live microorganisms

that, when administered in adequate amounts, confer health benefits on the host" (Saarela, 2000). Moreover, The term probiotic (mean for life and opposite of antibiotics) is relatively new and is currently used when we refer to bacteria associated with beneficial effects on humans and animals. The baises for assessing probiotic efficacy like tolerance to conditions of digestive tract, multiplicatin and operating capacity in the intestine, enhancement of immunue system functions, produce antibacterial factors, ability to colonise in the colon and improve the microbila balance and resistance to industerial prossesing conditions (FAO/WHO 2002 and Gomes *et al*., 2009). Isolation and screening of new probiotic strains of LAB from fermented milk products is highly interesting because of these microflora had a good technological and probiotic functions like organic acids production, proteolytic activity, exopolysaccharides production and antimicrobial activity against many food borne pathogens (El-Soda *et al*., 2003; Ayad *et al*., 2004; Abd El Gawad *et al*., 2010 and Ayad and Shokery, 2011). Recently, the use of functional starter cultures, a novel generation of starter cultures that offers functionalities beyond acidification, is being explored (Corsetti *et al*., 2012).The selection of probiotic bacteria include several criteria *i.e.* safety, viability, resistance to acid and bile salts, adherence to gut epithelial tissue, ability to colonize the gastrointestinal tract, production of antimicrobial substances, ability to stimulate a host immune response and the ability to influence metabolic activities such as vitamin production, cholesterol assimilation and lactose reduction (Tkhruni *et al*., 2013). There has been an increase of interest regarding the commercial utilization of *Lactobacillus* strains isolated from traditional and naturally fermented dairy products (Ambadoyiannis *et al*., 2015). Traditional Egyptian fermented dairy products such as Zabady (yoghurt), Laban Rayeb (concentrated sour milk); Karish cheese (skimmed milk cheese, and Kishk (wheat-based fermented milk) are a good valuable sources of LAB with new important technological, probiotic properties and genetic biodiversity (Abd El Gawad *et al*., 2010; El-Ghaish *et al*., 2010; Ayad and Shokery 2011 and Mabrouk *et al*., 2014). Therefore, the objective of the present work has been focused on screening the probiotic characteristics of some lactic acid bacteria were previously isolated from artisan Egyptian fermented dairy products.

Materials and Methods

Bacterial Strains

Bacterial strains were previously isolated and identified from dairy products and were kept in sterile reconstituted skim milk (12.5 % w/v) supplemented with 1% yeast extract and 25 % (w/v) glycerol then stored at -20°C in deep freezer until used.

Growth Medium and Chemicals

Columbia agar base, MRS broth and agar, bile salts, M17 broth and agar were purchased from (Oxoid)**,** Nutrient broth and agar were obtained from (Fluka), Human blood sample was obtained from Faculty of Medicine, Kasr El aini, Cairo, Egypt, Low heat skim milk powder extra grade (96.0% total solids) made in Australia was purchased from the local market.

Tolerance to Bile Salts

The method described by (Mabrouk *et al*., 2007) was used to determine the tolerance of examined lactic acid bacteria to bile salts. The strains were grown in MRS agar supplemented with different concentrations

of bile salts at the level of (0, 0.1, 0.3, 0.5, 0.7 and 1 % w/v). The viable cell counts (log cfu/ml) were determined and the plates were incubated anaerobically at 37°C for 48 h.

Low pH (High Acidity) Resistance

The examined lactic acid bacteria were evaluated for low pH according to the method described by (Jonganurakkum *et al*., 2008) was used to evaluate lactic acid bacteria for low pH resistance. One ml from each overnight pure examined strains in 9 ml MRS broth adjusted to pH 2 and pH 7.2 (control). The inoculated tubes were incubated at 37°C for various times (0, 1, 2, 3 and 5 h) then the viable bacterial cell counts (log cfu/ml) were determined in MRS agar. The plates were incubated anaerobically at 37°C for 48 h. All experiment was performed in three replicates.

Viability (%) of Strains in Different Concentrations of NaCl

The procedure of (Desai *et al*., 2004) was used to determine the tolerance of isolates to sodium chloride. All tested strains were grown in MRS agar supplemented with different concentrations of sodium chloride (0, 2, 4, 6, 8, and 10). The viable counts (log cfu/ml) of isolated strains were determined by using MRS or M17 agar and the viabilities (%) were also calculated as the following equation: Viability (%) = (log cfu/ml after 24 h / initial log cfu/ml) × 100.

Phenotypic Safety Assessment of Isolates

The haemolytic activities of isolated strains were determined according to (Marakoudakis *et al*., 2009) as follows: all examined strains were separately grown in MRS or M17 broth at 37°C for 24 h and then streaked onto Columbia agar base plates supplemented with 5 % (v/v) whole human blood. The plates were incubated anaerobically at 37°C for 48 h. then observed the clear zones and the color of haemolysis around the growth colonies. All experiment was performed in three replicates.

Results and Discussion

Properties of Isolated Lactic Acid Strains

A varitey of lactic acid bacteria are typically food grade and evaluated for their probiotic potential and were applied as adjunct cultures in various types of foods.

Tolerance to Bile Salts

As shown in figure 1 the bile tolerance of tested lactic acid strains were demonstrated variable susceptibility to bile salts concentrations. The survival (log cfu/ ml) of all examined were gradually decreased by increasing the concentrations of bile salts. The counts of all strains were gradually decreased in the survival from 4 to 5 log cycles. The high survivability was showed with the strains *Pediococcus pentosaceus* NRC AM4 (7.51) and *Lactococcus lactis ssp. lactis* NRC AM3 (7.31). The resistances to bile salts are different amongst the tested strains and the diversity in this study is in the harmony with those obtained by (Mishra and Prasad, 2005 and Murad and Nour-Eddine, 2006). Finally, the ability of strains may be due to the secretion enzymes which able to hydrolyze the bile salts and reduce the toxic effects on the growth of strains (Khalil *et al*., 2007 and Monteagudo-Meraa *et al*., 2012).

Resistance to Low pH (High Acidity)

The resistance of LAB to low pH is one of the major selection criteria for probiotic strains. [28]

The results in figure (2) showed that the survivals (log cfu/ml) of isolated lactic acid strains grown in modified MRS broth (pH 2) were enumerated in MRS agar after anaerobically incubation at 37 ^{o} C for 48 h. the survival of all tested strains under acidic conditions were decreased with increasing the incubation time and a wide changes in the survival (log cfu/ml) were observed after 3-5 hrs of exposure to pH 2. The highest survival 4.87 and4.73 was recorded with the strains *Lactococcus lactis* ssp. *lactis* NRC AM-3 and *Lb. pentosus* NRC AM-8 respectively.

Fig.1 Survival (log cfu/ ml) of Examined Strains Tested in MRS Medium Supplemented with Different Concentrations of Bile Salts

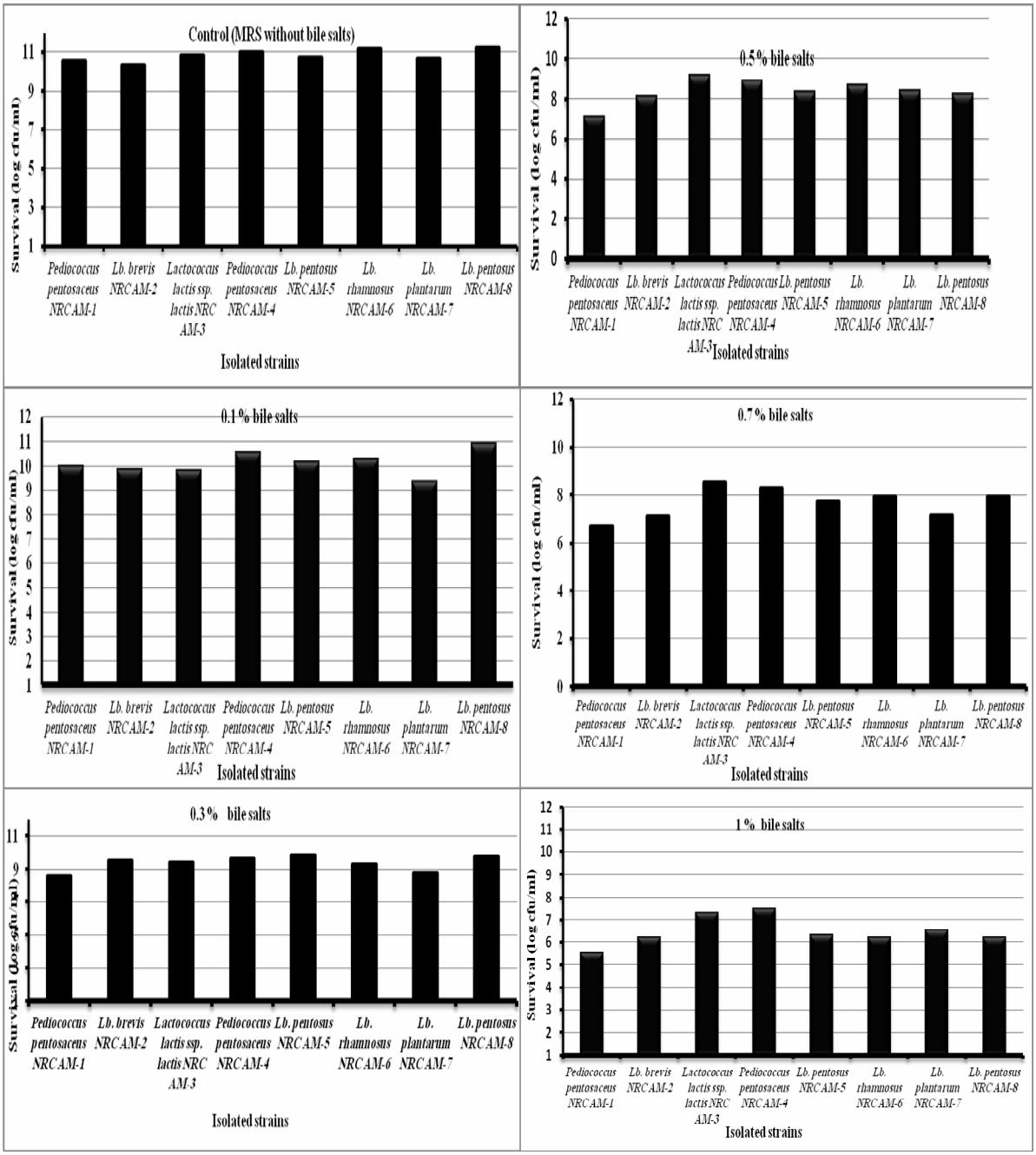


Fig.2 Effect of Low (pH 2) on Survival (log cfu/ml) of Examined Lactic Acid Strains

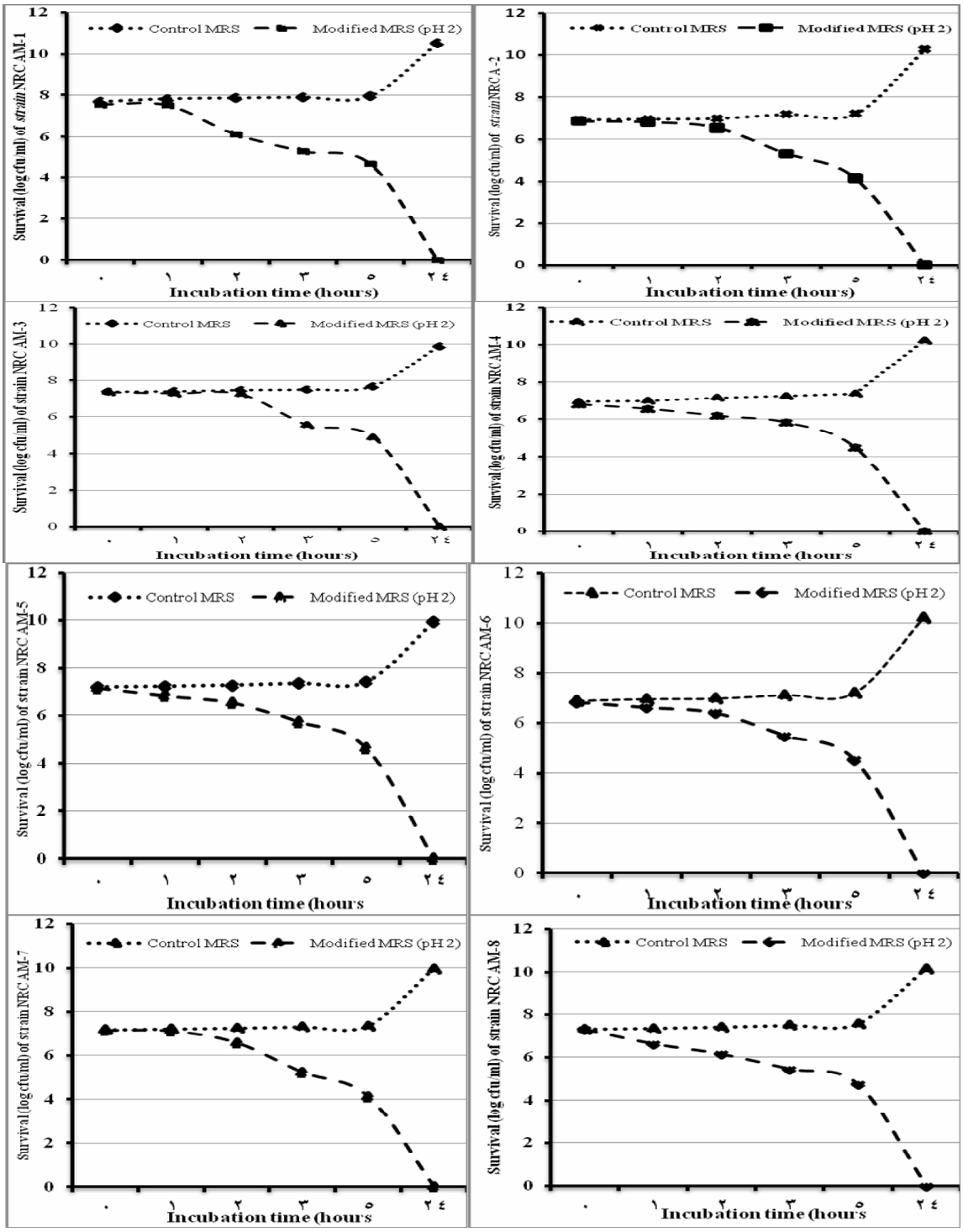
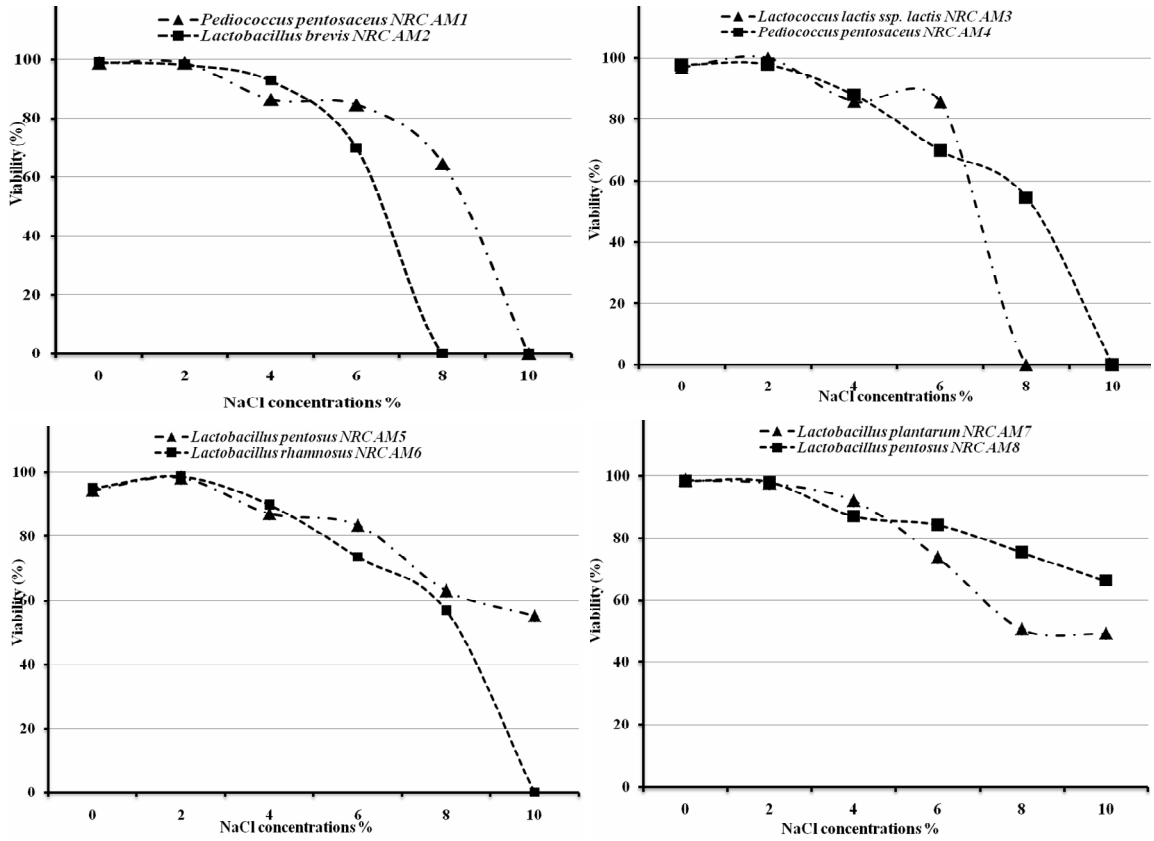


Fig.3 Viability (%) of Examined Strains Tested in MRS Medium Supplemented with Different Concentrations of NaCl after Incubation at 37° C for 24 h



Generally, all tested strains were affected by exposure to the pH 2 and there was not any growth after 24 h in all examined strains. After the examination of all tested strains, the strains that survive well in pH 2 were taken to use them as aciduric strains and good probiotic potential. The findings are in agreement with those reported by (Prasad *et al*., 1998; Mohammed *et al*., 2009; Lo Curto *et al*., 2011 and Pitino *et al*., 2012). They reported that the viability and survival of LAB examined at pH 2, 2.5 and 3.0 were decreased under the simulated gastric juice and most of strains are not able to survive at pH 2 except some *Lactobacillus* strains.

Viability (%) of Strains in Different Concentrations of NaCl

From the obtained results showed in figure 3. The viabilities % of all tested strains started high in MRS medium without NaCl (control) then gradually decreased as a result of increasing the concentration of NaCl to 10 % at the end of incubation period. The viability % of tested strains in MRS medium supplemented with 8 % NaCl decreased from (75.46) to (50.8) % with strains *Lb. pentosus* NRC AM8 and *Lb. plantarum* NRC AM7 respectively. The strain *Lb. brevis* NRC AM2 and *Lactococcus lactis* ssp. *lactis* NRC AM3 did not able to grow under this condition. Also, in the presence of 10 % NaCl the highest viabilities were recorded with strain *Lb. pentosus* NRC AM8 (66.18), strain *Lb. pentosus* NRC AM5 (64.47) and *Lb. plantarum* NRC AM7 (49.38). On the other hand strains *Pediococcus pentosaceus* NRC AM1, *Lb.*

brevis NRC AM2, *Pediococcus pentosaceus* NRC AM4, *Lb. rhamnosus* NRC AM6 and *Lactococcus lactis* ssp. *lactis* NRC AM3 could not be able to grow in the presence of 10 % NaCl. The findings are in the agreement with those obtained by (Ayad *et al*., 2004 and Mohammed *et al*., 2009).

Phenotypic Safety Assessment of Isolates

The haemolytic reactions were recorded by observation of a clear zone around the colonies (β-haemolysis), a partial hydrolysis and greening zone (α-haemolysis) or no reaction (γ-haemolysis). The results showed that all examined strains did not exhibit haemolytic activities (β-haemolysis) when grown in Columbia blood agar. Thus, these isolates have not exhibited any pathogenicity and regarded as safe organisms. These results are in the harmony with the earlier and many reports which are revealed that, LAB do not exhibit haemolysis and those obtained by (Malek *et al*., 2012; Vidhyasagar and Jeevaratnam, 2013 and Hawaz, 2014).

In Conclusion, The present study indicated that the novel lactic acid strains isolated from fermented dairy products have more effective functions. The examined strains for probiotic traits had a good ability to grow and survive well in the presence of different concentrations of bile salts (0.1, 0.2, 0.3 and 0.5 %) and gradually decreased from 4 to 5 log cycles with increasing the bile salts concentrations to 1 %. All tested strains were survived under acidic conditions (pH 2) till 5 hrs of incubation time at 37 ^{o}C but there was not any growth after 24 h. No haemolytic activities (β-haemolysis) were observed for all tested strains.

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