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The Characteristics of Locally Isolated *Lactobacillus acidophilus* and *Bifidobacterium infantis* Isolates As Probiotics Strains

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ABSTRACT

Two strains of *Lactobacillus acidophilus* and *Bifidobacterium infantis* isolated from breast-fed infants stool were tested to determine their suitability for use as probiotics by conducting a special protocol. The protocol includes the following tests: acid tolerance, bile tolerance, cholesterol assimilation, adhesion to the digestive system test and the test of their viability in the feed. Both isolates showed good acid resistance at pH of as low as 2, although the viable count was significantly decreased ($p<0.5$) from 7.65 ± 0.07 and 8.15 ± 0.05 at zero time to 7.22 ± 0.01 and 6.15 ± 0.21 after 60 minutes for *L. acidophilus* and *B. infantis*, respectively. The isolates could tolerate bile salt of 0.3 % concentration, although the viable counts decreased significantly ($p<0.5$) from 8.91 ± 0.03 and 9.52 ± 0.04 on 0% bile salt to 8.59 ± 0.01 and 8.69 ± 0.02 on 0.3% bile salt for *L. acidophilus* and *B. infantis*, respectively. Assimilated cholesterol was significantly higher ($p < 0.5$) for *L .acidophilus* (76.0 % ± 3.5) as compared with *B. infantis* (57.7 % ± 2.1). Both could adhere to rat intestine as the viable counts of third washing were 6.82 ± 0.05 and 7.85 ± 0.02 for *L. acidophilus* and *B. infantis*, respectively, as compared with the control (3.57 ± 0.04). Also, both isolates could stay viable in the rat diet, with the minimum acceptable level, for 2-3 days. On the basis of the results of this study, it can be concluded that both isolates have the characteristics of probiotics strains.

Keywords: Probiotics, Characterization of probiotics, *Lactobacillus acidophilus, Bifidobacterium infantis.*

INTRODUCTION

Widespread interest in the possibility that selected foods might promote health has resulted in the coining of the term ″functional foods″ (Milner, 2000). Probiotics and prebiotics, which may positively affect various physiological functions of the body, are among many food ingredients which can be classified as functional foods (Roberfroid, 2000).

The use of probiotics evolved from a theory proposed by the Nobel Prize–winning scientist Elie Metchnikoff, who suggested that the prolonged life span of Bulgarian peasants was a result of their consumption of fermented milk products (yoghurt containing lactobacilli) (Duggan *et al*., 2002), which eliminate putrefactive intestinal bacteria (Stanton *et al*., 2001). Several definitions of a probiotic have been suggested by different investigators. Probiotic has been defined by Fuller in 1989 as "a live microbial food supplement which beneficially affects the host animal by improving its microbial balance" (Goldin, 1998).

In the development of probiotic foods intended for human consumption, strains of lactic acid bacteria, such as *Lactobacillus* as well as *Bifidobacterium* and *Streptococcus*, have been used most commonly,

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primarily because they are desirable members of the intestinal microflora. In addition, these bacteria have traditionally been used in the manufacture of fermented dairy products and have GRAS (generally regarded as safe) status (Dunne *et al*., 2001).

Nutritional and health aspects of functional foods incorporating probiotic bacteria, especially lactic acid bacteria (LAB) and bifidobacteria, have received considerable attention, and eventually led to numerous claims in the literature (Gomes and Malcata, 1999). Therapeutically, probiotics have been used to modulate immunity, prevent cancer recurrence, prevent diarrhea (Sleator and Hill, 2008), treat rheumatoid arthritis and lower cholesterol, improve lactose intolerance, prevent or reduce Crohn's disease and prevent constipation as well as candidiasis and urinary tract infections (Reid, 1999).

The selection of the bacterial isolates to be used as effective probiotic strains is a complex process, especially as all of the features which an isolate should possess for maximum efficacy are not yet known. In general, the isolates should possess several physiological and biochemical criteria. These criteria may be summarized as follows: the bacteria should be of human origin, have a nonpathogenic behavior, be resistant to gastric acidity and bile toxicity, have an adhesion property to gut epithelial tissue, have the ability to colonize within the GI tract, produce antimicrobial substances, have the ability to degrade mucin (Delgado *et al.*, 2008) and have the ability to influence metabolic activities (eg., cholesterol assimilation, lactase activity and vitamin production) (Dunne *et al*., 2001).

This study aimed at studying the *in vitro* efficacy of *Lactobacilus acidophilus* and *Bifidobacterium infantis* local isolates as probiotic strains by applying a special protocol. These isolates were intended to be used later in a rat experiment as probiotic strains.

MATERIALS AND METHODS

Microorganism Isolation and Purification

Lactobacillus acidophilus (*L. acidophilus*) and *Bifidobacterium infantis* (*B. infantis*) isolates used in this research were previously isolated from the stools of new born breast-fed infants (Haddadin and Takruri, 2004). One gram of freeze-dried powder of each of these isolates was transferred aseptically into 50 mL sterile de Man, Rogosa and Sharpe (MRS) broth supplemented with 0.5% L(+)cysteine-HCl (99.6% purity, Sigma, USA), then incubated at 37 °C for 20 hours in an anaerobic jar (Oxoid, UK).

Repeated streaking onto MRS agar plates was used for purification for both isolates. One colony from each plate was picked up and inoculated into 5 mL sterile MRS broth. The isolates were activated by making subculturing twice in MRS broth containing 0.5% cysteine-HCl (Sigma, USA), as reducing agent, using 1% inoculum and 18 - 20 hours of incubation at 37°C in an anaerobic jar (Oxoid, UK).

Maintenance of Cultures

The two isolates were maintained by subculturing in MRS broth, containing 0.5% L(+)cysteine-HCl (Sigma, USA), using 1% inoculum and 18 - 20 hours of incubation at 37°C in an anaerobic jar (Oxoid, UK). The cultures were kept in the refrigerator at 4°C between subcultures. Each isolate was subcultured two to three times prior to every test (Walker and Gilliland, 1993).

Isolate Identification

The species of the two isolates (*L. acidophilus* and *B. infantis*) used in this study were confirmed physiologically and biochemically according to Bergey's Manual of Systematic Bacteriology (Kandler and Weiss, 1986) and Prokaryotes (Hammes *et al*., 1992; Biavati *et al*., 1992). Each culture was tested for Gram stain reaction, catalase production and the ability to grow at

15 and 45°C. The ability of cultures to produce ammonia from arginine, to hydrolyze esculin and to ferment 20 additional substrates were also tested. Fructose 6-phosphate phosphoketolase (Fructose-6-PP) test for the identification of *Bifidobacterium* was conducted (Biavati *et al*., 1992).

Preparation of the Supplementary Cultures

Liquid skim milk was used for both supplementary culture treatments. A 9% powder skim milk (Regilait, France) was reconstituted in distilled water, supplemented with 0.5% L(+)cysteine-HCl (Sigma, USA) and autoclaved at 115 °C for 10 minutes. After cooling, the milk was inoculated with 2% (v/v) of freshly prepared culture of each isolate alone and incubated at 37 °C for 16 hours under anaerobic conditions. The two supplementary cultures were prepared weekly and kept in the refrigerator to be used as supplementary cultures.

Characteristics of *L. acidophilus* and *B. infantis* Isolates as Probiotics

Tests applied to measure the ability of the isolates as probiotic strains included acid resistance, bile resistance, cholesterol assimilation, adhesion properties (Pereira and Gibson, 2002) and viability of the isolates in the food (Haddadin *et al*., 1997).

Acid Resistance

Isolates were tested for acid resistance as described by Pereira and Gibson (2002). MRS broth was adjusted to pH 2 using 10 N-HCl. A 10% inoculum (vol/ vol) from the third subculture of each isolates was inoculated into 10 mL MRS broth test tubes. Incubation was at 37°C for 2 hours in an anaerobic jar (Oxoid, UK). 0.1 mL samples from each test tube were taken at 0, 60 and 120 minutes, serially diluted 10-fold in an anaerobic diluent (peptone water plus 0.5 g of L-cysteine HCl /L), and plated in duplicate onto MRS agar. The plates were incubated at 37°C for 48 hours under anaerobic conditions before enumeration. Differences in counts were used to assess the acid resistance of the isolates. Strains with a final count more than $\log_{10}$ 4 were considered as acid tolerant.

Bile Resistance

The method of Haddadin *et al*. (1997) was used to assess the bile tolerance properties of the isolates. MRS broth containing 0.3% bile acids (Oxgall, Difco, USA), to mimic an approximate level of bile acids in the intestine, and 0.5% L(+)cysteine-HCl (Sigma, USA), as reducing agent, were used. Duplicate tubes of MRS broth (10 mL) containing 0 % and 0.3 % were inoculated with 0.1 mL of the test cultures and incubated in an anaerobic jar (Oxoid, UK) at 37 °C for 24 hours. Total viable counts of the test isolates were made by using MRS agar.

Cholesterol Assimilation

A modification of the Gilliland and Walker (1990) method was used to assess cholesterol assimilation capabilities of test cultures. Fresh sheep blood was used as a source of cholesterol. Blood samples were collected in a 500 mL sterile clean Duran bottle and immediately transferred under cold conditions to the laboratory. The blood was centrifuged at 3000 rpm for 15 minutes (Medifuge, Haereus, Germany), separated and micro-filtrated using a 0.22µm sterile filter (Satorious, GmbH, Germany).

MRS broth supplemented with 0.3% bile acids (Oxoid, UK) and 0.2% sodium thioglycollate (Sigma, USA), as a reducing agent, was sterilized at 121 °C for 15 minutes. After cooling, a 20% of sterile blood plasma was aseptically added (Haddadin *et al*., 1997). The MRS broth container was shaken several times to homogenize the contents. The broth was then aseptically dispensed (6 mL/tube) into sterile screw-cap test tubes. Two tubes were held as uninoculated controls, and the others were inoculated with 2% from the third subcultures of the test

isolates. All test tubes were incubated at 37°C under anaerobic conditions for 24 hours.

The culture cells were removed by centrifugation of the test tubes at 3000 rpm for 20 minutes under cold conditions, followed by microfiltration using 0.22µm sterile filter. Spent broth was collected and placed into clean and dry test tubes. An enzymatic-chromogenic method (Atlas, USA) was used to determine the cholesterol concentration in control and culture test tubes (Periera and Gibson, 2002). Differences in the amount of cholesterol between the control and the culture test tubes were considered as the assimilated amounts.

Adhesion Properties

A modification of Piette and Idziak (1992) was used to assess adhesion properties of the test isolates. Two slices of Sprague-Dawley rat small intestine per each isolate were cut and weighed, 2.0 g for each. The slices were soaked in 25 mL MRS broth, supplemented with 0.5% L(+)cysteine-HCl (Sigma, USA), containing bacterial suspension of the test isolates which had been incubated previously at 37 ºC for 16 hours under anaerobic condition. Two slices were held as control in MRS broth blank. All slices were left in MRS broth for 1 hour at 37ºC to initiate the adhesion process. Each slice were washed with 100 mL sterilized water 3 times for 5 minutes. Finally, each slice was held in 18 mL sterilized peptone water (1%) and homogenized by stomacher machine (AES, France). Total colony counts for the isolates in the macerate were determined.

Inability to Hydrolyze Mucin

The ability of the two bacterial isolates to hydrolyze mucin was tested based on Bergey's Manual of Systematic Bacteriology (Kandler and Weiss, 1986) where pig mucin was utilized.

Antipathogenicity of the Isolates

Eschericia coli (*E. coli*) enterohemorrhagic, *Salmonella typhi*, *Salmonella sonnei* and *Shigella dysentrea* from MUCL (Belgium) were used to test the antipathogenicity of the species according to Awaisheh (2003).

Viability of the Isolates in Experimental Rat Diets

The method of Haddadin *et al*. (1997) was used to assess the viability of the isolates in experimental rat diets (to be used in a paralel study). A 100 g sample of each rat experimental diets, to which probiotics should be added: (basal + probiotics), (basal + prebiotics + probiotics), (cholesterol + probiotics) and (cholesterol + prebiotics + probiotics) diets, were placed in separate beakers. Two percent (w/w) of liquid cultures of each *L.acidophilus* and *B. infatis* were added and mixed well with each sample. The beakers were placed in the animal unit lab at room temperature. A sample of 5 g diet of each beaker was taken at different intervals (0, 6, 12, 24, 48 and 72 hours), to determine the total viable count of the bacteria in the diet. The 5 g were transferred into 45 mL sterilized peptone water (1%), and the suspension was shaken using a shaker at 200 rpm for 15 minutes. After that, the total viable counts of both isolates were made using MRS agar.

Statistical Analysis

The statistical analyses were performed using the Statistical Analysis System (SAS, 1996). Analysis of variance (ANOVA) with Least Significant Difference test (LSD) was used to determine any significant differences between the means (Steel and Torrie, 1980). Values in tables are expressed as means ± standard deviation (SD).

RESULTS

Physiological and Biochemical Characteristics of the Isolates

Results of the physiological and biochemical tests are presented in Table (1). All these results were in

accordance with the main features described in Bergey's Manual of Systematic Bacteriology (Kandler and Weiss, 1986), Prokaryotes (Hammes *et al*., 1992; Biavati *et al*., 1992). From these results, the isolates were confirmed as *L. acidophilus* and *B. infantis*.

Characteristics of *L. acidophilus* and *B. infantis* Isolates as Probiotics

Acid Resistance

The acid resistance results of the two isolates, which were chosen as probiotic microorganisms, are shown in Table (2). After two hours, the maximum count was 5.70 (Log_{10} CFU/mL) for *L. acidophilus* isolate.

Bile Resistance

The results of bile salt resistance test to 0.3% are also shown in Table (2). The total viable counts of both isolates decreased with 0.3% (w/v) bile salt, as compared with the control (0% bile salt) of each isolate. The bile resistance percentages were 48.2% and 14.7% for *L. acidophilus* and *B. infantis*, respectively. This means that in the presence of 0.3% bile salts for 24 hours, 48.2% of the total count of *L. acidophilus* and 14.7% of the total count of *B. infantis* were not affected by the added bile salt.

Cholesterol Assimilation

Measurement results of the amount of cholesterol assimilated in MRS broth during 24 hours of growth of the isolates, incubated anaerobically at 37 °C, are presented in Table (2). *L. acidophilus* and *B. infantis* decreased significantly ($p< 0.05$) the level of cholesterol in MRS broth. The amounts of cholesterol assimilated were 76.0% and 57.7% of the total cholesterol added to the media for *L. acidophilus* and *B. infantis*, respectively.

Adhesion Properties

Results for total viable count of two isolates recovered from the rat small intestinal wall after 1 hour of anaerobic incubation and after 3rd washing, compared with those of the control, are shown in Table (2). These results show that both isolates can exceed the 3rd washing and can adhere to the intestinal wall.

Viability of the Isolates in the Feed

The results of the viability test of the two isolates added to the rats feed at different types of diet and time intervals are presented in Table (3). The viability of the two isolates in different diet types decreased with time, the counts of the *B. infantis* isolate (log_{10} CFU/g feed) reached the minimum acceptable level at 48 hours; while *L. acidophilus* counts reached the minimum acceptable level at 72 hours. Accordingly, the supplementary cultures of the two isolates were mixed with the experimental diets every two days, to ensure the viability of the isolates in the feed during the experimental study period.

DISCUSSION

Characteristics of *L. acidophilus* and *B. infantis* Isolates as Probiotics

Acid Resistance

Ingested probiotics are exposed during their transit through the GIT to successive stress factors that influence the survival of those microorganisms. Passing through the stomach, which has a pH as low as 1.5, is one hurdle that faces probiotics on their way to the intestines (Marteau *et al*., 1997). Probiotic bacteria must first survive transit through the stomach, before reaching the intestinal tract (Dunne *et al*., 2001). The food transit time through the human stomach is about 90 minutes. Accordingly, probiotic bacteria should be able to tolerate acid for at least 90 minutes (Chou and Weimer, 1999).

The effect of acidity on the viability of the two isolates is presented in Table (2). For each isolate, there is a significant variation in counts after 1 hour and after 2 hours of exposing to pH 2. These variations in acid tolerance are in agreement with the work of Chou and

Weimer (1999) and that of Pereira and Gibson (2002), where great variations of acid tolerance have been shown in both LAB and *Bifidobacteria* isolates.

In this study, *B. infantis* isolate was shown to be less resistant than *L. acidophilus* isolate. This is in agreement with Gomes and Malcata (1999), who mentioned that LAB are known to be more acid tolerant than *Bifidobacteria*. According to Pereira and Gibson (2002), both isolates, which have a final count of more than 10^4, were considered as acid-tolerant and would be expected to survive and pass the stomach.

Prescott *et al.* (1999) reported that microorganisms often adapt to pH changes through potassium/proton and sodium/proton antiport systems. Some bacteria synthesize an array of new proteins as a part of what has been called their acidic tolerance response. A proton-translocating ATPase contributes to this protective response, either by making more ATP or by pumping protons out of the cell. Chaperones, protein molecules, such as acid shock proteins are synthesized if the external pH decreased. These chaperones prevent the acid denaturation of proteins and aid in the refolding of denatured proteins. Also, some microorganisms make their environment more alkaline by generating ammonia through amino acid degradation.

Bile Resistance

Another factor that should be considered in selecting probiotic culture is bile resistance, which enables a selected strain to survive, grow and perform therapeutic benefits in the intestinal tract (Usman and Hosono, 1999).

Bile acids are synthesized from cholesterol and conjugated to either glycine or taurine in the liver, then stored in the gall bladder. They are secreted from the gall bladder into the duodenum in the conjugated form (500–700 ml/d). These acids then undergo extensive chemical modifications (deconjugation, dehydroxylation, dehydrogenation and deglucuronidation) in the intestine as a result of microbial activity (Dunne *et al*., 2001).

The bile stress for ingested microorganisms in the gastrointestinal tract (GIT) is complex because bile concentrations and residence times vary in each compartment of the GIT (Marteau *et al*., 1997). Gilliland *et al*. (1984) and Gilliland and Walker (1990) pointed out the importance of bile tolerance of probiotic strains used as dietary adjunct.

The effects of bile salt resistance are shown in Table (2). A variation in bile tolerance was observed between the two isolates. This variation was in agreement with that in the literature (Haddadin *et al*., 1997; Chou and Weimer, 1999; Pereira and Gibson, 2002).

The mechanism of bile acids resistance is not well-understood yet. Although, many reports indicated that the presence of bile salt hydrolase (BSH) enzyme is responsible for bacterial resistance to bile acids (Gilliland and Walker, 1990; Tanaka *et al*., 1999; Coroz and Gilliland, 1999). This enzyme has been detected in the gut microflora genera such as *Lactobacillus* and *Bifidobacterium* (Tanaka *et al*., 1999).

Cholesterol Assimilation

Cholesterol removal from media by probiotic bacteria is one of the most important factors in probiotic selection for use as a dietary adjunct with a hypocholesterolemic potential (Gilliland and Walker, 1990). The ability of *L. acidophilus* and *B. infantis* to reduce cholesterol in laboratory growth media, in the presence of bile and in the absence of oxygen, is in agreement with the previous works on LAB and *Bifidobacteria* done by Gilliland *et al*. (1985), Gilliland and Walker (1990), Klaver and van der Meer (1993), Tahri *et al*. (1995) and Pereira and Gibson (2002).

In comparison to results available in the literature, the two tested isolates can be considered good cholesterol assimilators. About 76% of the cholesterol (13.2 of 17.5 mg/dL) was assimilated by *L. acidophilus*.

On the other hand, *B. infantis* assimilated 57.7% of the cholesterol (10.0 of 17.5 mg/dL) (Table 2).

Different proposed mechanisms are involved in the cholesterol reduction from the laboratory media, including cholesterol assimilation (Gilliland *et al*., 1985), co-precipitation with deconjugated bile acids as a result of BSH activity (Klaver and van der Meer, 1993), both assimilation and co-precipitation with deconjugated bile acids (Tahri *et al*., 1995) or incorporation of cholesterol into cellular membrane (Noh *et al*., 1997). These mechanisms may explain the variation in the amount of assimilated cholesterol between the two tested isolates.

Inability to Hydrolyze Mucin

The two isolates have inability to hydrolyze pig mucin (Table 2) which is similar to the results obtained by Delgado *et al.* (2008).

Antipathogenicity of the Isolates

The two isolates showed strong antipathogenicity against different species of pathogenic bacteria (Table 2) which is similar to what has been reported by Awaisheh (2003) and in agreement with the findings of Delgado *et al*. (2008).

Adhesion Properties

Adhesion to gut epithelial tissue and the ability to colonize the gastrointestinal tract are considered important criteria for the selection of probiotic bacteria, to exert their functionality in the intestines such as cholesterol assimilation (Dunne *et al.*, 2001; Sanders and Klaenhammert, 2001). Adhesion was considered to have occurred if the viable count of tissue macerates exceeded that of the third washing of the tissue (Haddadin *et al.*, 1997).

The results of the adhesion ability test of the two isolates are presented in Table (2). Although there is a difference between the two isolates in the adhesion ability, the two isolates have a good adhesion ability to the wall of small intestine, since they can exceed the 3rd washing with water. These results are in agreement with those of Haddadin *et al*. (1997) who reported that *L. acidophilus* has a good adhesion ability.

The observed differences in adhesion results of the two isolates can be explained by many proposed mechanisms for the attachment of bacteria to epithelial cells, including the presence of fibrillae and lipoteichoic acids, amphiphilic compounds on the cell surface (Tannock, 1992). Cell-surface charge and hydrophobicity (Piette and Idziak, 1992), having a protein-mediated mechanism and carbohydrate moieties or both (Sanders and Klaenhammert, 2001) are of the proposed mechanisms. The adhesion of bacteria to mucus, a glycoprotein layer which covers intestinal epithelium cells, is also an important requirement for adhesion to epithelium cells (Ouwehand *et al*., 1999).

The difference in adhesion ability of the probiotic strains can be used to explain why certain strains have shown longer wash-out periods after cessation of probiotic intake, while other strains have shown shorter wash-out periods.

The two isolates were tested for their stability in terms of the above-mentioned characteristics in three consecutive sub-culturings. It was found that all of the tested characteristics remained constant. Therfore, inspite of the possibility of the development of mutations in bacterial isolates during repeated subculturing, it was shown that there is relative stability against mutational effect.

Viability of the Isolates in the Feed

The viability of the bacteria in the feed, before consumption, is a very important characteristic for the probiotic to reach the GIT with its minimum acceptable level ($\approx 10^6$ CFU/g). The aim of this test was to determine how long 2% (w/w) of each *L. acidophilus* and *B. infatis* isolates can survive in rat experimental diets.

The present study reveals that *L. acidophilus* isolate remained viable in all feed types at the minimum acceptable levels for 3 days while, *B. infantis* isolate remained viable in all feed types at the minimum acceptable levels for 2 days (Table 3). These differences in the CFU/g reduction between the two isolates are expected, since *L. acidophilus* is facultative anaerobe, while *B. infantis* is strictly anaerobe. The oxygen toxicity is of particular relevance to *Bifidobacteria* (Gardiner *et al*., 2002)

Because there is a difficulty in maintaining anaerobic conditions during supplying the bacterial cultures to the feed, it seems that the best method to maintain viable bacteria at the required levels is by continuous supplying of rats feeds with bacterial culture every two days. This is in agreement with Haddadin *et al.* (1997) work, who added *L. acidophilus* at a 2.0 % (wt/wt) to the layer hen feed every two days, to ensure the minimum acceptable level of *L. acidophilus* in the feed.

The present investigation demonstrated that first the viability of our isolates was achieved in dry diets up to three days. This is considered an excellent finding with good potential for use at the industrial scale. Second, the same isolates were found by Awaisheh *et al.* (2005) and Haddadin *et al.* (2004) to show viability over fifteen days in yoghurt without any adverse effects on flavors . Also, these isolates exhibited strong viability with honey (Haddadin *et al.*, 2007) and with propolis (Haddadin *et al.*, 2008) making them candidates for industrial application.

From the results of this study, it can be concluded that *L. acidophilus* and *B. infantis* isolates have the characteristics of probiotics and realize the different conditions of the probiotic protocol. These characteristics may be related to their intestinal origin.

Table(1): The physiological and biochemical reactions of *B. infantis* and *L. acidophilus*.

Test	Results			
	B. infantis	*Prokaryotes*	*L. acidophilus*	*Bergey's Manual*
Gram Stain	+	+	+	+
Catalase test	–	–	–	–
NH_3 production from Arginine			–	–
Growrh in Aerobic Condition	–	–		
Growth at 15ºC			–	–
Growth at 45ºC			+	+
Glucose (gas Production)	–	–	–	–
Acid production from				
Amygdaline			+	+
Arabinose	–	–	–	–
Cellobiose	–	–	+	+
Fructose	+	+	+	+
Galactose	+	+	+	+
Glucose	+	+	+	+
Gluconate	–	–		
Inuline	+	V		
Lactose	+	+	+	+
Maltose	+	+	+	+
Mannitol	–	–	–	–
Mannose	–	V	+	+
Melizitose	–	–	–	–
Melibiose	+	+	+	V
Raffinose	+	+	+	V
Rahmnose			–	–
Ribose	+	+	–	–
Starch	–	–		
Salicin	–	–	+	+
Sorbitol	–	–	–	–
Sucrose	+	+	+	+
Trehalose	–	–	+	+
Xylose	–	V	–	–
Hydrolysis of Esculin			+	+
Fructose-6-PP	+	+		

+ =positive, - = negative, V = positive or negative.

Table (2): Probiotic characteristic of the *L. acidophilus* and *B. infantis* isolates.

Isolates	***L. acidophilus***	***B. infantis***
Tested characteristics		
1) Effect of acidity (pH 2) at different times[1,2]:	Viable count (log CFU/ mL)	
0 min	7.65[a] ± 0.07	8.15[a] ± 0.05
60 min	7.22[b] ± 0.01	6.15[b] ± 0.21
120 min	5.70[c] ± 0.06	5.20[c] ± 0.16
2) Effect of bile acid[1, 2, 3]:	Viable count (log CFU/ mL)	
0 %	8.91 [a] ± 0.03	9.52 [a] ± 0.04
0.3 %	8.59 [b] ± 0.01	8.69 [b] ± 0.02
Bile resistance %	48.2	14.7
3) Cholesterol assimilation[4, 5, 6]:		
mg/ dL	13.2 [a] ± 0.64	10.0 [b] ± 0.35
%	76.0[a] ± 3.5	57.7 [b] ± 2.1
4) Mucin hydrolysis	-ve	-ve
5) Antipathogenicity[7]	+ve	+ve
6) Adherence property[1,8]	Viable count (log CFU/ mL) after 3 [rd] washing	
	6.82 [b] ± 0.05	7.85 [c] ± 0.02

1. Each value is represented as a mean ± SD of duplicate readings.

2. Means with different superscripts within the same column are significantly different ($p < 0.05$).

3. Bile resistance% = no. of CFU/ml of the isolate in MRS broth with 0.3% bile salt ÷ no. of CFU/ml of the isolate in MRS broth with 0% bile salt × 100.

4. Average initial cholesterol concentration in the media was 17.5 mg/dl, which was considered as a control.

5. Each value is represented as a mean ± SD of triplicate readings.

6. Means with different superscripts within the same raw are significantly different ($p < 0.05$).

7. Antipathogenicity against *E. coli* enterohemorrhagic, *Salmonella typhi, Salmonella sonnei and Shigella dysentrea.*

8. Means with different superscripts within the same raw are significantly different ($p < 0.05$) compared to the control which contains 3.57 [a] ± 0.04 CFU/ mL.

Table (3): Viability of *L. acidophilus* and *B. infantis* (log_{10} CFU/g feed) in different types of diet and time intervals[1, 2]

Diets / Time (hours)	Diet 1 Basal+Probiotics			Diet 2 Basal+Probiotics+Prebiotics		
	L. acidophilus	***B. infantis***	**Total count**	***L. acidophilus***	***B. infantis***	**Total count**
0	$7.59^{a} \pm 0.02$	$7.71^{a} \pm 0.03$	$7.96^{a} \pm 0.01$	$7.51^{a} \pm 0.04$	$7.80^{a} \pm 0.04$	$7.98^{a} \pm 0.01$
6	$7.29^{b} \pm 0.04$	$7.37^{b} \pm 0.01$	$7.63^{b} \pm 0.03$	$7.32^{b} \pm 0.03$	$7.45^{b} \pm 0.05$	$7.69^{b} \pm 0.01$
12	$7.10^{c} \pm 0.08$	$7.22^{b} \pm 0.02$	$7.47^{c} \pm 0.02$	$7.19^{c} \pm 0.01$	$7.36^{b} \pm 0.03$	$7.59^{c} \pm 0.01$
24	$6.87^{d} \pm 0.12$	$6.99^{c} \pm 0.06$	$7.25^{d} \pm 0.02$	$7.04^{d} \pm 0.06$	$7.08^{c} \pm 0.05$	$7.36^{d} \pm 0.02$
48	$6.65^{e} \pm 0.07$	$6.59^{d} \pm 0.15$	$6.93^{e} \pm 0.04$	$6.82^{e} \pm 0.05$	$6.74^{d} \pm 0.06$	$7.06^{e} \pm 0.03$
72	$6.30^{f} \pm 0.01$	$5.84^{e} \pm 0.08$	$6.43^{f} \pm 0.03$	$6.54^{f} \pm 0.08$	$5.90^{e} \pm 0.07$	$6.63^{f} \pm 0.06$

Diets / Time (hours)	Diet 3 Cholesterol+Probiotics			Diet 4 Cholesterol+Probiotics+Prebiotics		
	L. acidophilus	***B. infantis***	**Total count**	***L. acidophilus***	***B. infantis***	**Total count**
0	$7.49^{a} \pm 0.04$	$7.78^{a} \pm 0.01$	$7.96^{a} \pm 0.02$	$7.54^{a} \pm 0.01$	$7.83^{a} \pm 0.03$	$7.99^{a} \pm 0.01$
6	$7.21^{b} \pm 0.04$	$7.47^{b} \pm 0.03$	$7.66^{b} \pm 0.01$	$7.32^{b} \pm 0.06$	$7.46^{b} \pm 0.07$	$7.70^{b} \pm 0.14$
12	$7.04^{b\,c} \pm 0.06$	$7.20^{c} \pm 0.08$	$7.43^{c} \pm 0.07$	$7.24^{b\,c} \pm 0.06$	$7.34^{b} \pm 0.06$	$7.59^{c} \pm 0.01$
24	$6.88^{c\,d} \pm 0.04$	$6.93^{d} \pm 0.04$	$7.21^{d} \pm 0.04$	$7.12^{c} \pm 0.05$	$7.13^{c} \pm 0.07$	$7.42^{d} \pm 0.06$
48	$6.65^{d} \pm 0.07$	$6.54^{e} \pm 0.08$	$6.90^{e} \pm 0.01$	$6.84^{d} \pm 0.08$	$6.74^{d} \pm 0.11$	$7.09^{e} \pm 0.02$
72	$6.15^{e} \pm 0.21$	$5.80^{f} \pm 0.14$	$6.33^{f} \pm 0.10$	$6.54^{e} \pm 0.08$	$5.78^{e} \pm 0.01$	$6.61^{f} \pm 0.06$

1. Each value is represented as a mean ± SD of duplicate readings.

2. Means with different superscripts within the same column are significantly different ($p < 0.05$).

REFERENCES

Awaisheh, S. (2003). *In vitro* studies of the effect of fermented dairy products containing probiotics and neutraceuticals on different characteristtics of normal flora. Ph.D. Thesis, the University of Jordan, Amman, Jordan.

Awaisheh, S., Haddadin, M.S.Y. and Robinson, R.K. (2005). Incorporation of selected neutraceuticals and probiotic bacteria into fermented milk. ***Intern. Dairy J.,*** 15:1184-1190.

Biaviati, B., Sgorbati, B. and Scardovi, V. 1992. The genus *Bifidobacterium*, In: The prokaryotes, a handbook on the biology of bacteria: ecophysiology, isolation, identification, applications, (2nd ed.), edited by Balows, A. *et al.* Springer-Verlag, New York, 1535-1573.

Chou, L-S. and Weimer, B. 1999. Isolation and characterization of acid- bile-tolerant isolation from strains of *Lactobacillus acidophilus*. ***J. Dairy Sci.,*** 82: 23 -31.

Corzo, G. and Gilliland, S. E. 1999. Bile salt hydrolase activity of three strains of *Lactobacillus acidophilus*. ***J. Dairy Sci.,*** 82: 472 -480.

Delgado, S., Sullivan, E., Fitegerald, G. and Mayo, B. 2008. *In vitro* evaluation of the probiotic properties of human intestinal bifidobacterium species and selection of new probiotic candidates. ***J. Appl. Microb.***, 104: 1119-1127.

de Roos, N. M. and Katan, M. B. 2000. Effects of probiotic bacteria on diarrhea, lipid metabolism and carcinogenesis: A review of papers published between 1988 and 1998. ***Am. J. Clin. Nutr.***, 71 (2): 405 -411.

Duggan, C., Gannon, J. and Walker, W. A. 2002. Protective nutrients and functional foods for the gastrointestinal tract. ***Am. J. Clin. Nutr.***, 75: 789 -808.

Dunne, C. L., O'Mahony, L. M., Thornton, G., Morrissey, D., O'Halloran, S., Feeney, M., Flynn, S., Fitzgerald, G., Daly, C., Kiely, B., O'Sullivan, G., Shanahan, F. and Collins, J. K. 2001. *In vitro* selection criteria for probiotic bacteria of human origin: Correlation with *in vivo* findings. ***Am. J. Clin. Nutr.***, 73 (2): 386S -392S.

Gardiner, G. E., Ross, P., Kelly, P. M., Stanton, C., Collins, J. K. and Fitzgerald, G. 2002. Microbiology of therapeutic milks. In: Dairy Microbiology Handbook, (3rd ed.), edited by Robinson R. K. Wiley-Interscience, USA, pp. 431 -478.

Gilliland, S. E. and Walker, D. K. 1990. Factors to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. ***J. Dairy Sci.,*** 73: 905 -911.

Gilliland, S. E., Nelson, C. R. and Maxwell, C. 1985. Assimilation of cholesterol by *Lactobacillus acidophilus*. ***Appl. Environ. Microbiol.,*** 49 (2): 377 -381.

Gilliland, S. E., Staley, T. E. and Bush, L. J. 1984. Importance of bile tolerance of *Lactobacilli acidophilus* used as a dietary adjunct. ***J. Dairy Sci.,*** 67: 3045 -3051.

Goldin, B. R. 1998. Health benefits of probiotics. ***Br. J. Nutr.,*** 80 (2): 203S –207S.

Gomes, A. M. P. and Malcata, F. X. 1999. *Bifidobacterium* spp. and *Lactobacillus acidophilus*: Biological, biochemical, technological and therapeutical properties relevant for use as probiotics. ***Trends in Food Sci. and Tech.,*** 10: 139 -157.

Haddadin M.S.Y, Nazer, I., Abu-Raddad, J.I. and Robinson, R.K. 2007. Effect of honey on the growth and metabolism of two bacterial species of intestinal origin. ***Pakistan J. Nutr.***, 6 (6): 693-697.

Haddadin, M. Y. S. and Takruri, H. R. 2004. Production of health food project, The University of Jordan, Department of Nutrition and Food Science, Financed by the Higher Council for Science and Technology. (Personal Communication).

Haddadin, M. Y. S., Abdulrahim, S. M., Odetallah, N. H.

and Robinson, R. K. 1997. A proposed protocol for checking suitability of *Lactobacillus acidophilus* cultures for use during feeding trials with chickens. ***Trop. Sci.,*** 37: 16 -20.

Haddadin, M.S.Y., Nazer, I., Abu-Raddad, S.I. and Robinson, R.K. 2008. Effect of probiotics on bacterial species with probiotic potential. ***Pakistan J. Nutr.***, 7(2): 391-394.

Haddadin, M.S.Y., Awaisheh, S. and Robinson, R.K. 2004. The production of yoghurt with probiotic bacteria isolated from infants in Jordan. ***Pakistan J. Nutr.***, 3(5):290-293.

Hammes, W. P., Weiss, N. and Holzapfel, W. 1992. The genera *Lactobacillus* and *Carnobacterium*. In: The prokaryotes, a handbook on the biology of bacteria: ecophysiology, isolation, identification, applications, (2nd ed.), edited by Balows, A., *et al.* Springer-Verlag, New York, 535-1573.

Kandler, O. and Weiss, N. 1986. Regular, nonsporing gram-positive rods. In: Bergey's manual of systematic bacteriology, Vol. 1, edited by Krieg, N. *et al*. Baltimore, MD: Williams and Wilkins Co., 1208 -1234.

Klaver, F. A. M. and van der Meer, R. 1993. The assumed assimilation of cholesterol by *Lactobacilli* and *Bifidobacterium bifidum* is due to their bile salt-deconjugating activity. ***Appl. Environ. Microbiol.,*** 59 (4): 1120 -1124.

Marteau, P., Minekus, M., Havenaar, R. and Veld, J. H. J. 1997. Survival of lactic acid bacteria in a dynamic model of the stomach and small intestine: validation and the effects of bile. ***J. Dairy Sci.,*** 80: 1031 -1037.

Milner, J. A. 2000. Functional foods: The US perspective. ***Am. J. Clin. Nutr.***, 71 (6): 1654S-1659S.

Noh, D. O., Kim, S. H. and Gilliland, S. E. 1997. Incorporation of cholesterol into the cellular membrane of *Lactobacillus acidophilus* ATCC 43121. ***J. Dairy Sci.,*** 80: 3107 -3113.

Ouwehand, A. C., Kirjavainen, P. V., Gronlund, M. M., Isolauri, E. and Salminen, S. J. 1999. Adhesion of probiotic micro-organisms to intestinal mucus. ***Inter. Dairy J.***, 9: 623 -630.

Pereira, D. I. and Gibson, G. R. 2002. Cholesterol assimilation by lactic acid bacteria and *Bifidobacteria* isolated from the human gut. ***Appl. Environ. Microbiol.,*** 68 (9): 4689 -4693.

Piette, J. P. G. and Idziak, E. S. 1992. A Model study of factors involved in adhesion of *Pseudomonas fluorescens* to Meat. ***Appl. Environ. Microbiol.,*** 58 (9): 2783 -2791.

Prescott, L. M., Harley, J. P. and Klein, D. A. 1999. Microbiology, (4th ed.), McGraw-Hill, New York.

Reid, G. 1999. The scientific basis for probiotic strains of *Lactobacillus*. ***Appl. Environ. Microbiol.,*** 65 (9): 3763 -3766.

Roberfroid, M. B. 2000. Prebiotics and probiotics: Are they functional foods? ***Am. J. Clin. Nutr.***, 71(6): 1682S -1687S.

Sanders, M. E. and Klaenhammer, T. R. 2001. Invited review: The scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. ***J. Dairy Sci.,*** 84: 319 -331.

SAS. 1996. Institute Inc., Version 6.12

Sleater R.D. and Hill, C. 2008. New fronteirs in probiotic research. ***Lett. Appl. Microb.***, 46:143-147.

Stanton, C., Gardiner, G., Meehan, H., Collins, K., Fitzgerald, G., Lynch, P.B. and Ross, R. P. 2001. Market potential for probiotics. ***Am. J. Clin. Nutr.***, 73 (2): 476S –483S.

Steel, R. G. D. and Torrie, J. H. 1980. Principles and procedures of statistics: a biometric approach, (2nd ed.), McGraw- Hill, New York.

Tahri, K., Crociani, J., Ballongue, J. and Schneider, F. 1995. Effect of three strains of *Bifidobacteria* on cholesterol. ***Lett. Appl. Microb.***, 21: 149 -151.

Tanaka, H., Doesburg, K., Iwasaki, T. and Mierau, I. 1999. Screening of lactic acid bacteria for bile salt hydrolase activity. ***J. Dairy Sci.,*** 82: 2530 -2535.

Tannock, G. W. 1992. The lactic microflora of pigs, mice and rats. In: The lactic acid bacteria in health and disease, (Vol.1), edited by Wood B. J. B., Elsevier Applied Science, New York, 21-48.

Usman, M. and Hosono, A. 1999. Bile tolerance, taurocholate deconjugation and binding of cholesterol by *Lactobacillus gasseri* Strains. ***J. Dairy Sci.,*** 82: 243 -248.

Walker, D. E. and Gilliland, S. E. 1993. Relationship among bile tolerance, bile salt deconjugation and assimilation of cholesterol by *Lactobacilli acidophilus*. ***J. Dairy Sci.,*** 76: 956 -961.

خصائص سلالتين من بكتيريا *لاكتوباسيلاس أسيدوفيلاس وبيفيدوباكتيريوم انفانتس* معزولتين محلياً كسلالات للمحفزات الحيوية

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ملخـص

تم فحص سلالتين من بكتيريا *لاكتوباسيلاس أسيدوفيلاس و بيفيدوباكتيريوم انفانتس*، معزولتين من براز أطفال رضعوا رضاعة طبيعية، لتحديد ملاءمة استخدامهما كسلالات للمحفزات الحيوية (probiotics) و ذلك باتباع طريقة فحص خاصة. شملت طريقة الفحص هذه: فحص القدرة على تحمل الحموضة و فحص القدرة على تحمل أملاح المرارة و فحص استهلاك الكوليسترول والالتصاق بجدران أمعاء الجرذان والقدرة على الحياة في الغذاء. وقد أظهرت السلالتان قدرة جيدة على تحمل حموضة برقم هيدروجيني (pH) منخفض هو (2.0)، وذلك على الرغم من انخفاض العدد الكلي معنوياً ($p<0.5$) على هذا الرقم من 7.65 ± 0.07 و 8.15 ± 0.05 إلى 7.22 ± 0.01 و 6.15 ± 0.21 بعد 60 دقيقة من بداية التجربة لكل من عزلتي *لاكتوباسيلاس أسيدوفيلاس* و *بيفيدوباكتيريوم انفانتس*، على التوالي. وكانت العزلتان قادرتين على تحمل تركيز لأملاح المرارة مقداره 0.3%، مع أن العدد الكلي للبكتيريا انخفض معنوياً ($p<0.5$) من 8.91 ± 0.03 و 9.52 ± 0.04على تركيز 0% من أملاح المرارة إلى 8.59 ± 0.01 و 8.69 ± 0.02 على تركيز 0.3 و ذلك لكل من عزلتي *لاكتوباسيلاس أسيدوفيلاس وبيفيدوباكتيريوم انفانتس*، على التوالي. أما نسبة استهلاك الكوليسترول فكانت أعلى معنوياً ($p<0.5$) لعزلة *لاكتوباسيلاس أسيدوفيلاس* (76.0 % ±3.5) مقارنةً بعزلة *بيفيدوباكتيريوم انفانتس* (57.7% ± 2.1). وقد تبين أن السلالتين كانتا قادرتين على الالتصاق بجدران أمعاء الجرذان وقادرتين على الحياة في غذاء الجرذان (بأقل مستوى مقبول) لمدة 2-3 أيام. ويمكن بالاعتماد على النتائج المذكورة أعلاه استنتاج أن عزلتي *لاكتوباسيلاس أسيدوفيلاس وبيفيدوباكتيريوم انفانتس* لهما خصائص سلالات المحفزات الحيوية.

الكلمات الدالة: المحفزات الحيوية، خصائص المحفزات الحيوية، *لاكتوباسيلاس أسيدوفيلاس، بيفيدوباكتيريوم إنفانتس.*

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