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Probiotic Characteristics of Some Bifidobacteria and Leuconostoc Strains and Growth Behavior of the Selected Strains with Different Prebiotics.

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ABSTRACT

The present study aims to evaluate the probiotic potential of some lactic acid bacteria and select the candidates to be used as probiotic bacteria with some prebiotics in the functional foods. Ten of Bifidobacteria and Leuconostoc strains (8 Bifidobacteria and 2 Leuconostoc) were screened for their probiotic potential. The *in vitro* tests included survival in simulated gastrointestinal tract conditions (low pH, pepsin, bile salts, and phenol), resistance to 6 antibiotics and antimicrobial activity (against *Escherichia coli* 0157: H7 ATCC 6933, *Bacillus cereus* ATCC 33018, *Staphylococcus aureus* ATCC 20231, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella typhimurium* ATCC 14028 and *Listeria monocytogenes* V7 serotype 1). Five selected strains were *B. adolescentis* a-1, *B. longum* BB-536, *B. longum* b-1, *B. bifidum* Bb 12 and *L. mesenteroides subsp. mesenteroides* B-512f demonstrated the highest final population (≥ 8 log cfu/ml) after exposure to MRSc broth (pH 3) for 3 hrs or when grown in MRSc medium with 0.3% pepsin at pH 3 and after incubation for 24 hr in MRSc medium with 3% bile salts or incubation for 48 hr in MRSc medium with 0.3% phenol. All tested strains exhibited high resistance to four antibiotics (e. g. Amikacin, Norfloxacin, Cefadroxil and Cefoperazone). The other antibiotics, Rifampicin and Oxytetracycline inhibited all five selected strains. *Leu. mesenteroides subsp. mesenteroides* B-512f had the lowest sensitivity to both Oxytetracycline and Rifampicin compared to the other five selected strains. The cell free supernatant of *B. adolescentis* a-1 retarded growth of all pathogenic indicators whereas, *B. longum* b-1 and *Leu. mesenteroides subsp. mesenteroides* B-512f retarded growth of all pathogenic indicators except *Staphylococcus aureus* ATCC 20231 and *Salmonella typhimurium* ATCC 14028 respectively. *B. bifidum* Bb 12 retarded growths of all pathogenic indicators except *Staphylococcus aureus* ATCC 20231 and *Salmonella typhimurium* ATCC 14028 while *B. longum* BB-536 retarded growth *Escherichia coli* 0157: H7 ATCC 6933 and *Listeria monocytogenes* V7 serotype 1 only. The aforementioned five strains stimulated with Inulin and Fructooligosaccharide at 5% for each, while Lactulose was stimulant for *B. adolescentis* a-1 and *Leu. subsp. mesenteroides* B-512f without the others strains. Therefore, these strains could be selected for use as probiotics with Inulin or Fructooligosaccharide as prebiotics in functional foods.

Keywords: Probiotic; *Bifidobacteria*; *Leuconostoc*; prebiotic; generation time.

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INTRODUCTION

The balance and composition of the intestinal microbiota are important for the well-being and the ability of our organism to resist the invasion of pathogens. To increase the natural resistance of the host to infections, probiotic microorganisms can be used.

Probiotics have been defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2006). When ingested, these bacteria must overcome the gastrointestinal tract (GIT) barrier in sufficient numbers to arrive in the colon or in a metabolically active state, in order to transitorily persist in this environment, thus being able to exert their healthy effects. The extremely low pH (ranging from 1.5 to 3) and gastric enzymes in the stomach, followed by the bile salts, pancreatin and other intestinal enzymes that bacteria find in the duodenum, are the main challenges for probiotics (Masco *et al.,* 2007).

Bifidobacteria are one of the common probiotic bacteria of the intestinal tract (Cronin et al., 2011). They are helpful in maintaining a proper balance in the human intestinal flora, playing a protective role against potential pathogens and putrefactive bacteria; furthermore other health benefits such as, improved protein and vitamin metabolisms; prevention of constipation; treatment of liver damage; anti-tumor activity; stimulation of immune system response; reduction of blood cholesterol levels and improvement of lactose digestibility metabolisms (Chou and Weimer, 1999; Roy, 2001 and Mugueraza *et al.,* 2006)**,** hence they have been included in the probiotics group (Biavati *et al.,* 2000).

In dairy technology, the importance of Leuconostoc strains is widely recognized, related to numerous positive aspects such as, their role in the formation of aroma and texture of certain dairy products as non-starter lactic acid bacteria (NSLAB) in the same way as mesophilic lactobacilli (Cogan, 2002) and their role in functional foods as potential probiotics. As some microorganisms currently proposed to the consumers, Leuconostoc does not colonize the intestinal tract, and their effect on the host through microbial actions is thus expected to be small except when ingested at high cell concentrations.

Prebiotics stimulate the immune system (Schley and Field, 2002), protect the body from cancer (Klinder *et al.,* 2004), stimulate mineral absorption and bone stability (Scholz-Ahrens *et al.,* 2002) and treat irritable bowel-associated diarrhoeas (Cummings and Macfarlane, 2002). Prebiotics are utilized by the intestinal microbial population to produce short-chain fatty acids which may lead to the reduced incidence of gastrointestinal disease (Topping and Clifton, 2001), cancers (Hinnebusch *et al.,* 2002) and cardiovascular diseases (Dewailly *et al.,* 2001); and improvement of lipid profiles (Wolever *et al.,* 2002). Fructooligosaccharides, inulin, oligofructose, lactulose, and galactooligosaccharides have been identified as prebiotics due to characteristics such as resistance to gastric acidity, hydrolysis by mammalian enzymes and are fermented by gastrointestinal microflora to further selectively stimulate the growth and/or activity of beneficial intestinal bacteria (Gibson and Fuller, 2000). Because of these properties, fructooligosaccharides, inulin, oligofructose, lactulose, and galacto-oligosaccharides have recently received much attention as functional food ingredients (Oliveira *et al.,* 2011; Donkor *et al.,* 2007; Jyoti *et al.,* 2004 and Bruno *et al.,* 2002).

Therefore, the objectives of this study were to test ten strains from Bifidobacteria and Leuconostoc species to probiotic characteristics (e.g. acid tolerance, bile salt tolerance, phenol resistance, antibiotic resistance and antibacterial activity) and observation the growth behavior of selected strains when grown as single culture in MRSc medium supplemented with different concentrations of inulin, fructo- oligosaccharide and lactulose for selecting the best probiotic strains and the suitable prebiotic used in making the symbiotic fermented dairy products.

MATERIALS AND METHODS

Bifidobacteria; Leuconostoc strains and indicator pathogenic bacteria and culture conditions

Eight strains of Bifidobacteria , two strains of Leuconostoc and Indicator pathogenic bacteria were obtained from the sources indicated in Table (1).

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Table (1): source of microbiological cultures.

Strain	Source
B. adolescentis a-1 *B. longum* a-2 *B. longum* BB-536 *B. longum* b-1 *B. breve* 116 *B. breve* 1695 *B. bifidum* Bb 12	Dept. of Food Chem., Turku Univ., Finland
B. Bifidum B3 *Leu. mesenteroides subsp. cremoris* a-22	Dairy Microbiology Lab., National Res. Centre, Dokki, Giza, Egypt
Leu. mesenteroides subsp. mesenteroides B-512f	Microbial Properties Res. Unit, National Center for Agric. Utilization Res., USDA, Peoria, Illinois
Escherichia coli 0157: H7 ATCC 6933 *Bacillus cereus* ATCC 33018 *Staphylococcus aureus* ATCC 20231 *Pseudomonas aeruginosa* ATCC 9027 *Salmonella typhimurium* ATCC 14028	Agric. Res. Center , Giza, Egypt
Listeria monocytogenes V7 serotype 1	Dept. of Food Sci., Wisconsin Univ., Madison, USA

Bifidobacteria and Leuconostoc strains were activated separately in Oxoid MRSc broth medium containing 0.05% L-Cystein- HCl (MRSc) (Dave and Shah, 1996).Cultures were incubated anaerobically using Gas packs (anaerobic system, Oxoid) for 18 – 24 hrs at 37ºC without agitation (Dave and Shah, 1996). Between transfers the cultures were stored at 4ºC. Stock culture was prepared by cultivation of 0.5 ml pure culture in 2 ml MRSc broth medium to contain 2% glycerol at 37ºC and for 18 – 24 hrs then immediate freezing and storing at - 16ºC until experimental use (Van Den Berg *et al.,* 1995). Before use, the stock culture was activated by three successive transfers. The indicator pathogenic bacteria were stocked on Tryptone Soya agar medium (Oxoid) and propagated (37ºC for 24 hrs) in sterile Tryptone Soya broth medium (Oxoid).

Acid, simulated gastric juice, Bile salt, Phenol tolerance and Antibacterial activity

Erlenmeyer flasks containing 50 ml of MRSc broth medium were inoculated with cultures of tested strains separately, at ratio of 1% (v/v) and incubated at 37ºC for 24 hrs. The cells were harvested by centrifugation (3500 xg) at 4ºC for 10 min. To measuring acid tolerance, the cell pellets were washed in phosphate-saline buffer (PBS) and then resuspended in the same buffer. Phosphate-saline buffer cells suspension (0.5 ml) was diluted to 5 ml with sterile MRSc and pH was adjusted to 1, 2 or 3 by addition of 5 M HCl. The suspensions were incubated at 37ºC and viable organisms were enumerated after 0, 1, 2 and 3 hrs on MRSc agar medium (Minelli et al., 2004). To measuring simulated gastric juice tolerance, the cell pellets were suspended in 2 ml of fresh MRSc broth medium containing 0.3% pepsin. Cell suspension (0.5 ml) was diluted to 5 ml with sterile MRSc and pH was adjusted to 1, 2 or 3 with 5 M HCl. The suspensions were incubated at 37ºC and viable organisms were enumerated after 0, 1, 2 and 3 hrs on MRSc agar medium (Lian *et al*., 2003). To measuring bile salt tolerance, the cell pellets were resuspended in 2 ml of fresh MRSc broth medium and immediately pour plated in MRSc agar medium containing various bile concentrations (0, 0.5, 1, 1.5, 2, 2.5 and 3% (w/v) oxgall, Difco (Zinedine and Faid, 2007). To measuring phenol resistance, the cell pellets were resuspended in fresh MRSc broth medium at ratio of 1% (v/v) and immediately pour plated in MRSc agar medium containing various phenol concentrations (0, 0.1, 0.2, 0.3, 0.4 and 0.5% (Maria *et al*., 2006). All plates were anaerobically incubated at 37ºC for 48 hrs using Gas packs (Dave and Shah, 1996). To antibacterial activity test, cell free supernatant fluid, that resulted from harvesting of cells by centrifugation (5000 xg) at 4ºC for 10 min, was tested against the indicator pathogenic bacteria strains using agar diffusion well assay (Zinedine and Faid, 2007) but without neutralization.

Antibiotic resistance

The antibiotic sensitivity of the examined strains was tested according to the disc assay method (Mulamattathel *et al.,* 2000) using six antibiotic standard discs (Oxoid). The tested antibiotics were Amikacin (30 µg), Norfloxacin (10 µg), Cefadroxil (30 µg), Rifampicin (30 µg), Cefoperazone (75 µg) and Oxytetracycline (30 µg).

Growth behavior of selected Bifidobacteria and Leuconostoc strains

Among ten strains, the best five strains were selected according to their best probiotic characteristics. Growth behavior of these strains was observed when grown as single in MRSc broth medium supplemented with 1, 3 and 5% (w/v) of inulin, fructooligosaccharide (FOS) or lactulose as prebiotic. Cultures were incubated anaerobically using Gas packs for 24 hrs at 37ºC without agitation (Shin *et al.,* 2000). Viable organisms were enumerated on MRSc agar medium every 3 hrs for 24 hrs. All plates were anaerobically incubated at 37ºC for 48 hrs using Gas packs (Dave and Shah, 1996). Specific growth rate (µ) and generation time (T_g) of Viable organisms was calculated as follows (Oliveira *et al.,* 2011):

$$\mu = (\log_{10} X_2 - \log_{10} X_1) / (t_2 - t_1),$$

WhereX_2 and X_1 are the maximum count and the minimum count ($cfu.mL^{-1}$) at the end time = t_2 and zero time = t_1 $T_g = Ln (2/\mu)$

Statistical analyses

SAS software Version 7 (1996) was used to conduct statistical analyses, the one-way ANOVA test was used and the differences among means of treatments were compared by Duncan's test at significant (p=0.05).

RESULTS AND DISCUSSION

Acid tolerance

To assess whether Bifidobacteria and Leuconostoc strains could pass the gastrointestinal tract successfully, the tolerance to low pH *in vitro* was regarded as an important standard. But pH value of gastric juice went up and down depending on the difference of diet components. Generally, pH value of gastric juice in stomach is 0.9; however, the presence of food raises the pH value to the level of pH 3.0 and food transition time through the human stomach is about 90 min (Erkkia and Petaja, 2000 and Gopal *et al.,* 2001).

Figs (1 and 2) shows the survival of the tested Bifidobacterium and Leuconostoc cells after suspended them in MRSc broth with different pH values (pH 2.0 and 3.0) and incubated at 37ºC for 0, 1, 2 and 3 hrs to simulate low-acid conditions of human gastrointestinal tract. Although all 10 strains of Bifidobacteria and Leuconostoc had acid tolerance to some extent under exposed acid conditions (pH 2.0 and 3.0), different strains showed different survivability.

At pH 2.0, the viable enumerations of *B. breve* 116, *B. adolescentis* a-1, *B. longum* b-1, *Leu. mesenteroides subsp. mesenteroides* B-512f, *B. longum* BB-536 were above 9 log cfu/ml at 0 hrs, the rest of them were less than 9 log cfu/ml. After exposure for 1 hr, survival rates of some strains such as *B. longum* a-2 and *Leu. mesenteroides subsp. cremoris* a-22, were markedly increased (22.8 and 12.5 %, respectively), whereas survivability of other strains was remained as it is as *B. breve* 116, *B. bifidum* Bb 12 and *B. breve* 1695 or slightly decreased as *B. longum* BB-536, *B. longum* b-1, *B. bifidum* B3 and *Leu. mesenteroides subsp. mesenteroides* B-512f (Fig 1). When extension the incubation time up to 2 hrs at such acidic conditions all strains lose their viability. But at pH 3.0, all strains exhibited high tolerance even after 3 hrs incubation. For instance, the viable enumerations of three strains namely *B. adolescentis* a-1, *B. longum* BB-536 and *B. longum* b-1 were approached 10 log cfu/ml while other strains such as *Leu. mesenteroides subsp. mesenteroides* B-512f, *B. bifidum* Bb 12 and *Leu. mesenteroides subsp. cremoris* a-22, their viable enumeration was equal to 9 log cfu/ml after 3 hrs incubation (Fig 2). These results are in agreement with those reported by Ziyu *et al.,* (2007), Fayed *et al.,* (2008) and (Argyri *et al*., 2013).

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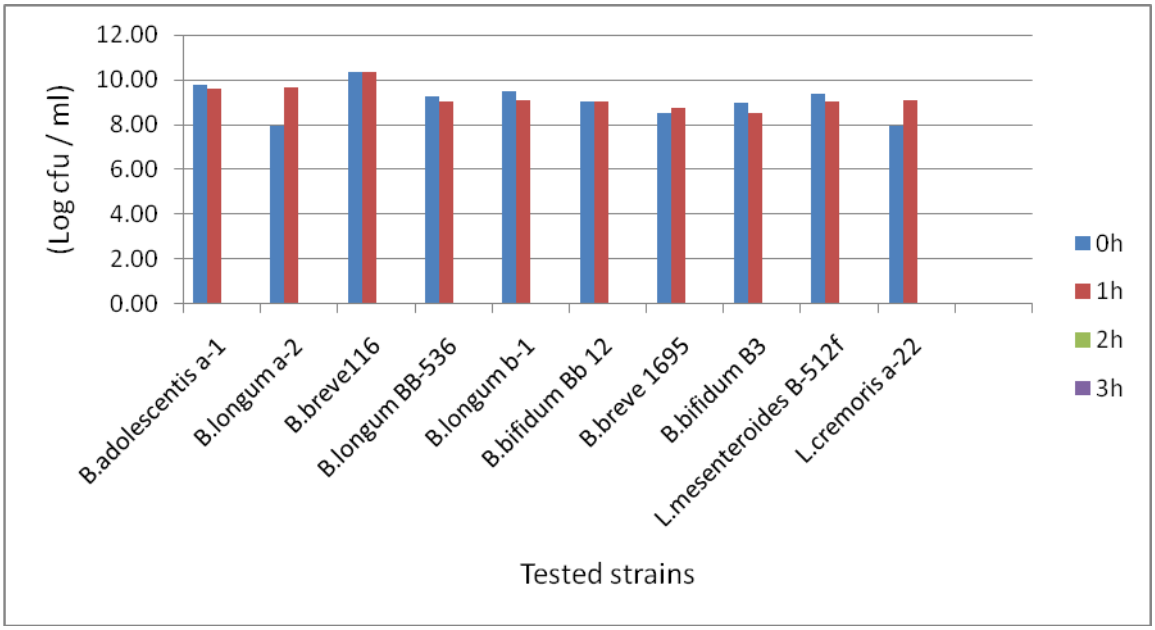

Fig (1) Survival of tested strains (Log cfu /ml) tested in MRSc broth at pH 2 during incubation at 37°C for 3 hr.

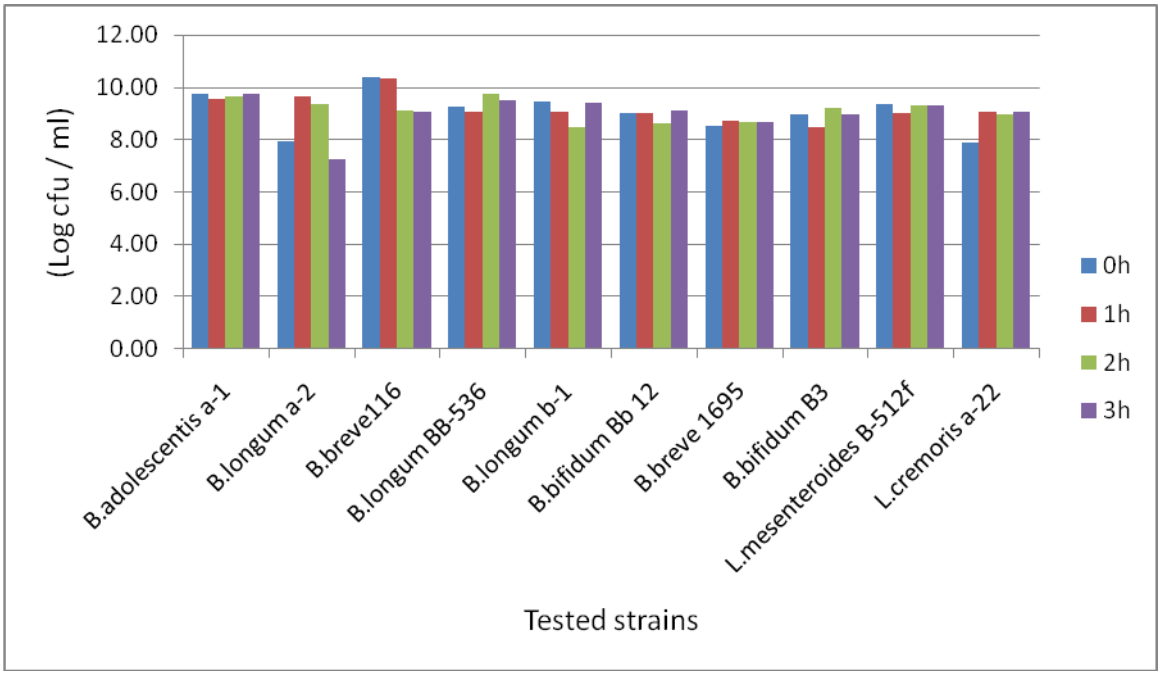

Fig (2): Survival of tested strains (Log cfu /ml) tested in MRSc broth at pH 3 during incubation at 37°C for 3 hr.

Simulated gastric juice tolerance

In order to exert the function of Bifidobacteria or Leuconostoc strains, it was important for them to survive in inhospitable environment of human gastrointestinal tract. Therefore, measuring their capacity of pepsin tolerance in simulated gastric juice at pH 2 and 3 with 0.3 % pepsin was necessary.

According to Figs (3 and 4) it could be concluded that the pepsin capability of ten strains were completely different. At pH 2.0 + 0.3% pepsin, one strain *B. longum* a-2 demonstrated better tolerant ability among ten strains and its survival rate was 96.6% after 1 hr exposure. Other strains such as *B. breve* 1695 and *B. bifidum* B3 showed good tolerant ability and their survival rates were 85.3 and 79.4%, respectively, while survival rates of *B. bifidum* Bb 12, *B. adolescentis* a-1 and *B. longum* BB-536 were 66.18, 60.1 and 53.3%, respectively, under same conditions. Cells of *B. breve* 116, *B. longum* b-1, *Leu. mesenteroides subsp.*

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mesenteroides B-512f and *Leu. mesenteroides subsp. cremoris* a-22 could not survive in MRSc broth with 0.3% (w/v) pepsin at pH 2.0, 37ºC for any exposure period. In addition to, all strains die with extending the exposure period up to 3 hrs (Fig. 3). At pH 3.0 + 0.3% pepsin, all strains remained alive until the end of third hour of the exposure (Fig. 4). The viable enumerations of *B. bifidum* B3, *Leu. mesenteroides subsp. mesenteroides* B-512f and *Leu. mesenteroides subsp. cremoris* a-22 were remained still during exposure periods (above 9 log cfu/ml) while decreased of other strains such as *B. bifidum* Bb 12, *B. longum* b-1, *B. longum* BB-536, *B. adolescentis* a-1, *B. longum* a-2 and *B. breve* 116 after exposure to 0.3% pepsin (pH 3.0) for 3 hrs, but with low rate to become their survival rates 97.8, 97.7, 92.0, 90.9, 89.7 and 88.6%, respectively.

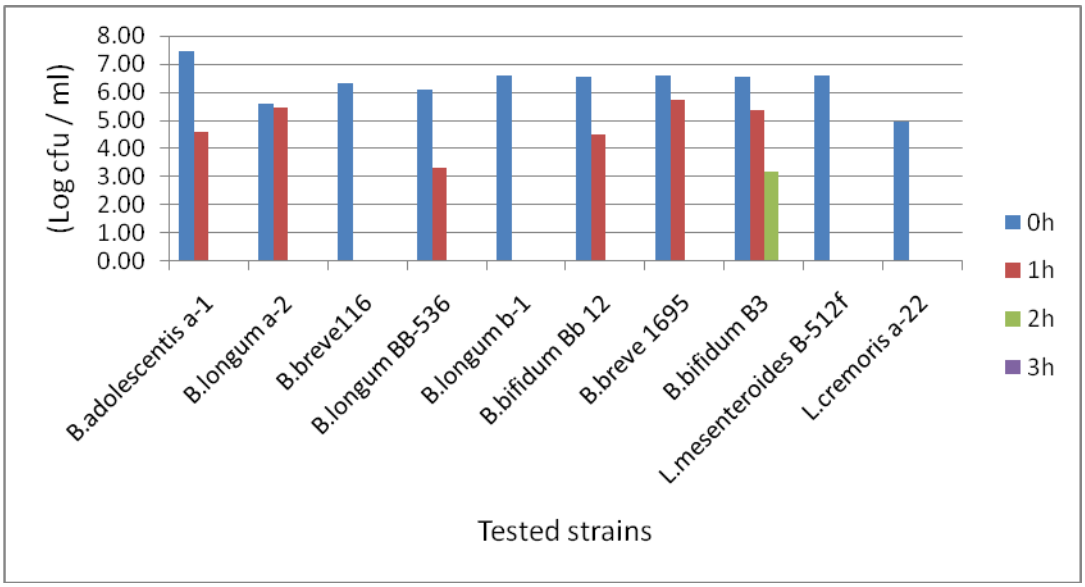

Fig (3): Survival of tested strains (Log cfu /ml) in MRSc broth at pH 2+0.3% Pepsin after incubation at 37°C for 3 hr.

Unusual, *B. breve* 1695 that its viable enumeration increased from 7.8 log cfu/ml at zero time to 9.1 log cfu/ml after 3 hrs in MRSc broth with 0.3% (w/v) pepsin at pH 3.0, 37ºC (Fig. 4). These results are in agreement with those proved by Ziyu *et al.,* (2007) who screened to 38 Bifidobacterium strains to probiotc properties. Among these strains, they selected six strains, *B. breve* A04 was the best. *B. breve* A04 had better survival capability to 0.5% pepsin (w/v) or 1% pancreatin (w/v) than other Bifidobacteria, and viable bacteria were above 8.00 log cfu/ml after incubation for 24 h.

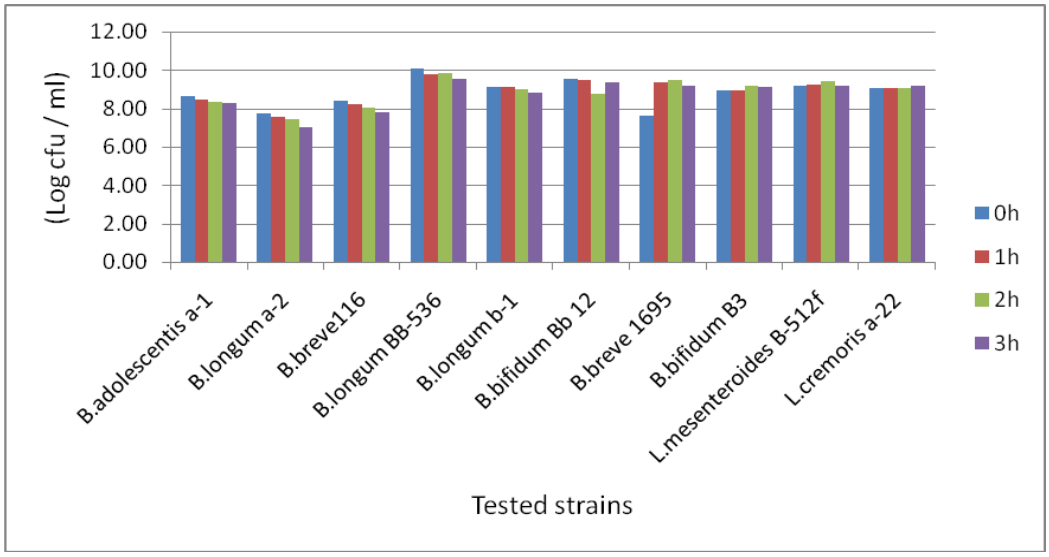

Fig (4): Survival of tested strains (Log cfu /ml) in MRSc broth at pH 3+0.3% Pepsin after incubation at 37°C for 3 hr.

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Bile tolerance

All tested Bifidobacteria and Leuconostoc strains showed significant decrease in viable cell numbers when grown in MRSc agar medium containing various bile concentrations 0.5, 1, 1.5, 2, 2.5 and 3% (w/v) in comparison with control medium (Fig. 5). The percentage of this decrease at 3 % bile salt was lowest of *B. bifidum* Bb 12 (19.3 %) follow by *B. bifidum* B3 and *Leu. mesenteroides subsp. cremoris* a-22 (22.83 % of each), then *B. longum* BB-536 and *B. breve* 1695 (26.35 % of each) while, reached its highest point of *B. longum* a-2 (35.13 %). *B. longum* a-2 was more sensitive than other strains to all bile salt concentrations used (Fig. 5). However, most strains such as *B. bifidum* Bb 12, *B. breve* 116, *Leu. mesenteroides subsp. cremoris* a-22 and *B. bifidum* B3 showed normal growth at bile concentrations of up to 1.5 % w/v. These findings are in agreement with those obtained by Sanders *et al.,* (1996) who found that most of the Bifidobacteria strains were resistant to bile concentrations varying from 1 to 3%. On the contrary, Erkkia and Petaja, (2000) concluded that the concentration of 0.1% and 0.2% bile salts had little influence to all Bifidobacteria strains tested; whereas, 0.3% bile salts was critical concentration for screening tolerant strains. This property may provide these strains with an advantage *in vivo*.

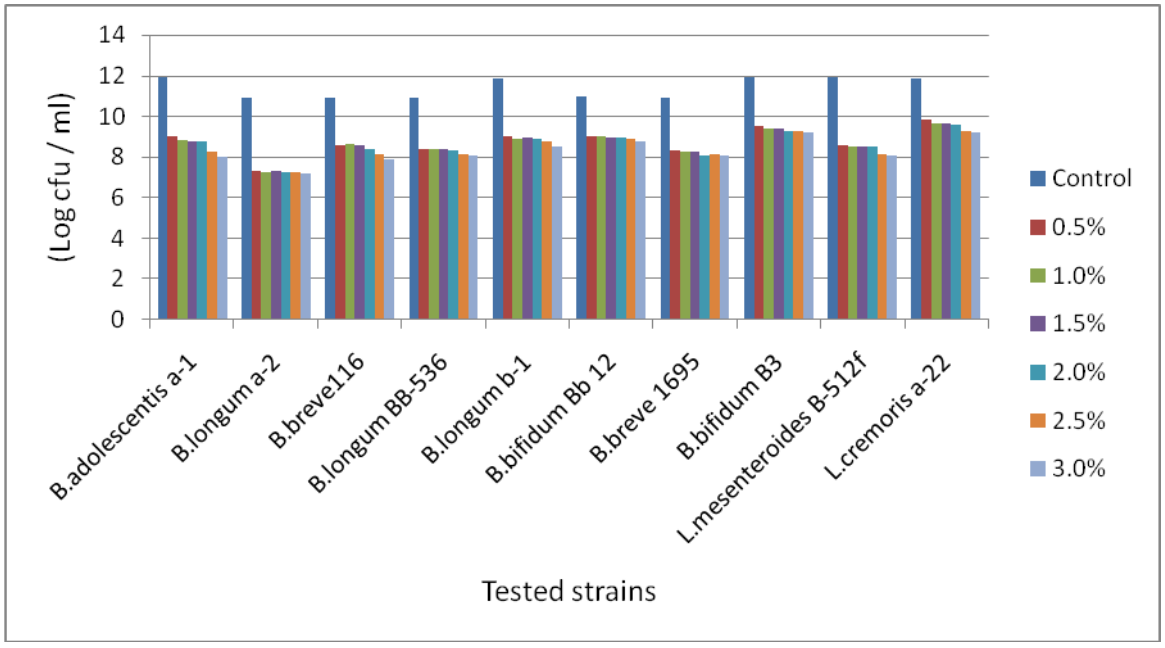

Fig (5): Survival of tested strains (Log cfu /ml) at different concentrations of bile salts after incubation at 37°C for 48 hr.

Phenol tolerance

Phenol is an intermediate of putrefactive processes in the intestinal tract and may be formed by the bacterial deamination of some aromatic amino acids derived from dietary or endogenous proteins (Suskovic *et al.,* 1997). Therefore, probiotics must be able to survive at phenol concentrations ranged from 0.3 to 0.6% w/v (Rajiv *et al.,* 2002).

Fig. (6) shows the survival of the tested Bifidobacteria and Leuconostoc strains at different concentrations of phenol in comparison with control. It can be noticed that viable cells count of all strains significantly decreased with increasing the phenol concentration to 0.4 and 0.5%. But *Leu. mesenteroides subsp. mesenteroides* B-512f was the most resistance to phenol at 0.4% whereas its viable cells count reached 9.41log cfu/ml after 48 hr in MRSc medium containing 0.4% phenol at 37ºC. At 0.5% phenol, the tested strains were arranged according to phenol resistance as follow: *B. longum* b-1 > *B. longum* BB-536 > *Leu. mesenteroides subsp. cremoris* a-22 > *B. breve* 116 > *B. adolescentis* a-1 = *B. breve* 1695 > *B. longum* a-2 = *B. bifidum* Bb 12 = *B. bifidum* B3 = *Leu. mesenteroides subsp. mesenteroides* B-512f, whereas, viable cells count of the first strain was 7.85 log cfu/ml and of the last four strains was ≈ 4.67 log cfu/ml after 48 hr in MRSc medium containing 0.5% phenol at 37ºC (Fig. 6).

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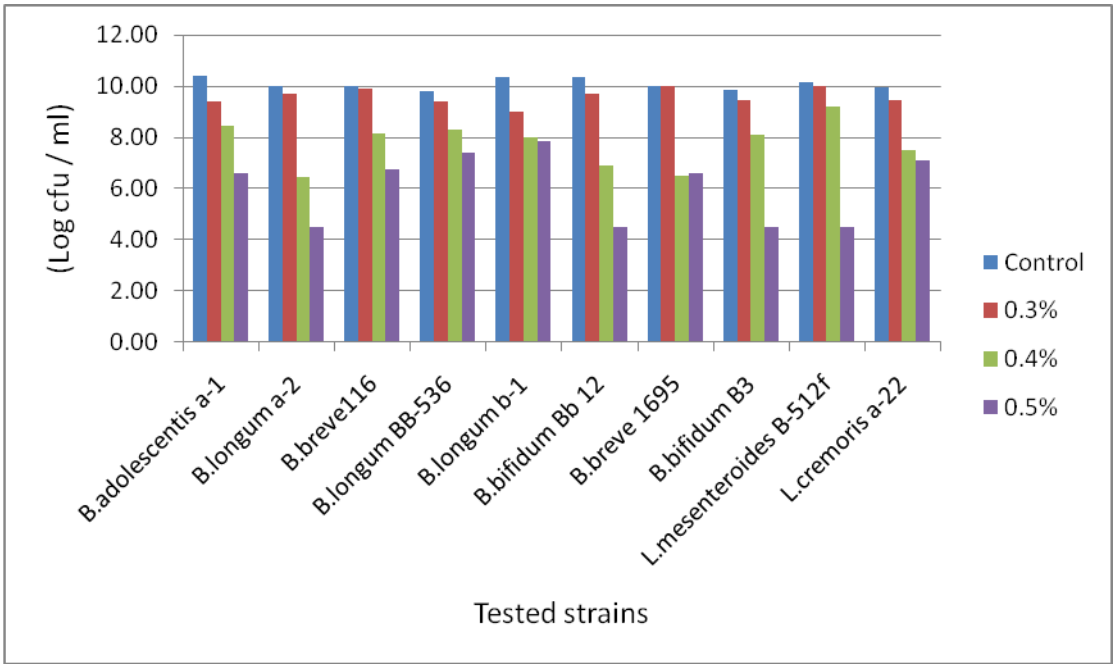

Fig (6): Survival of strains (Log cfu /ml) tested in MRSc medium at different concentrations of phenol after incubation at 37°C for 48 hr.

At 0.3% phenol, all strains exhibited a good phenol resistance whereas, viable cells count of the more sensitive strain (e.g. *B. longum* b-1) reached to 9.18 log cfu/ml after 48 hr in MRSc medium containing 0.3% phenol at 37ºC (Fig. 6). Consequently, this concentration (0.3%) has been used in most studies for screening phenol resistance (Rajiv *et al.,* 2002 and Fayed *et al.,* 2008).

Antibacterial activity

Antagonistic action could be due to lactic acid, hydrogen peroxide, bacteriocins, or combination of two or more of these factors (Kimoto *et al.,* 2000 and Ehrmann *et al.,* 2002).

Bacteriocins are antimicrobial proteins that are usually associated with organic compounds secreted by gram (+) bacteria (Jack *et al*., 1995 and Kotelnikova and Gelfand, 2002). During the last years several papers were published on bacteriocins produced by lactic acid bacteria (LAB). Compounds capable to inhibit the growth of potentially pathogenic species such as *L. monocytogenes*, *B. cereus*, *clostridium perfrigens* and some strains of *Staphylococcus aureus* were described (Lasagno *et al*., 2002 and Ross *et al*., 1999); they seem to exert a bactericidal more than a bacteriostatic effect. Bacteriocins are sensitive to proteolytic enzymes, but resistant to low and high pH values, to heat and to organic solvents. They are mainly produced during the early stationary phase; their synthesis is associated to a plasmid encoded gene and it can be described as a strain-specific characteristic (Yildirim *et al*., 1999).

The ability of cell free supernatant of each tested Bifidobacteria or Leuconostoc strain to restrain the growth of some pathogenic indicator bacteria is presented in Fig. (7). Among the tested strains, *B. breve* 116 not exhibited any antibacterial activities (e.g. no inhibition zone, I.Z.) against the used pathogenic indicators while *B. adolescentis* a-1 retarded growth of all pathogenic indicators but with different rates. Only three strains, *B. adolescentis* a-1 (inhibition zone diameter, I.Z.D. = 33 mm), *Leu. mesenteroides subsp. mesenteroides* B-512f (I.Z.D.= 25 mm) and *B. bifidum* B3(I.Z.D. = 17 mm) retarded growth *Staphylococcus aureus* ATCC 20231 while, *Salmonella typhimurium* ATCC 14028 was inhibited by *B. breve* 1695(I.Z.D. = 24 mm) and *B. longum* b-1(I.Z.D. = 13 mm) as well as *B. adolescentis* a-1(I.Z.D. = 11 mm). No inhibition zone could be detected using culture supernatant of both *B. breve* 116 against *Listeria monocytogenes* V7 serotype 1and *Escherichia coli* 0157: H7 ATCC 6933, *B. breve* 116 and *B. longum* BB-536 against *Bacillus cereus* ATCC 33018 and *B. breve* 116, *B. longum* BB-536 and *B. bifidum* B3 against *Pseudomonas aeruginosa* ATCC 9027 (Fig. 7). Unusual, *Leu. mesenteroides subsp. cremoris* a-22 had the highest antibacterial activities (I.Z.D.= 31 mm) against *Bacillus cereus* ATCC 33018 and was the first strain in this respect, while, *Leu. mesenteroides subsp.*

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mesenteroides B-512f caused the biggest inhibition zone against *Pseudomonas aeruginosa* ATCC 9027 compared with the other strains (Fig. 7). These findings are in agreement with those obtained by Fayed *et al.,* (2008) who found that *B. longum* Bb46 exhibited antibacterial activities against all the pathogenic indicator bacteria followed by *B. adolescentis* ATCC 15704. Kim *et al*. (2001) showed that the soluble substances present in the supernatant of *B. longum* strains reduce the effect of *E. coli* cytotoxins on mice by means of intestinal receptor protection. This observation represents an additional point in order to propose Bifidobacteria for technological and therapeutically applications.

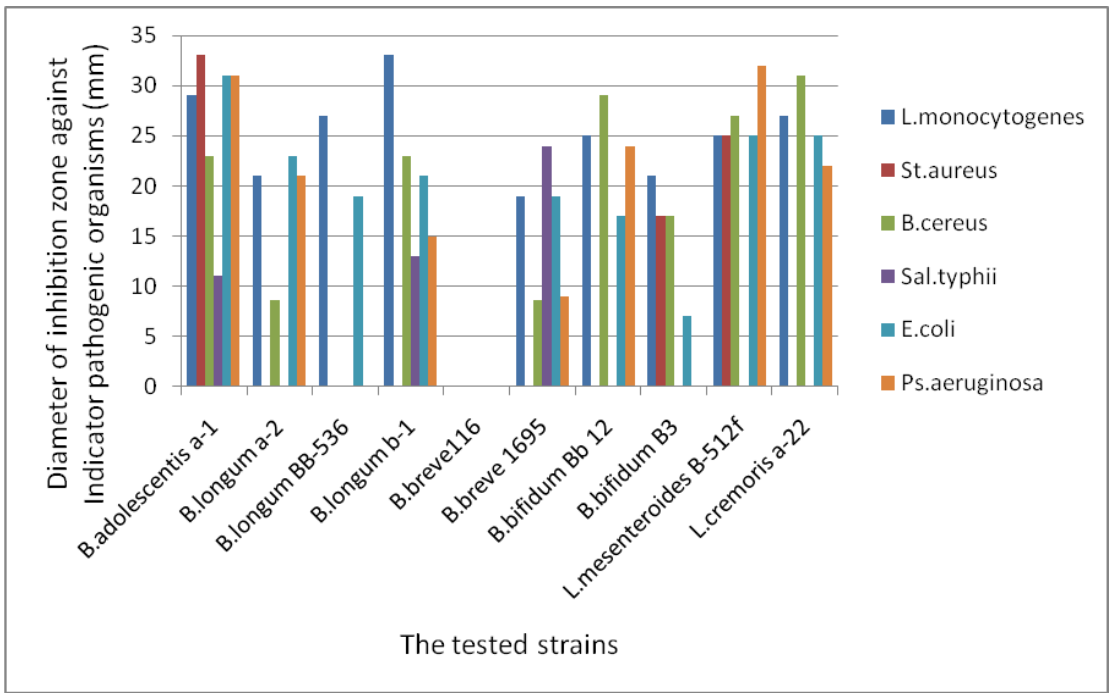

Fig (7): Antibacterial activity of tested strains of Bifidobacteria and Leuconostoc against some indicator pathogenic organisms measured by diameter of inhibition zone (mm).

Antibiotic resistance

Table (2): Antibiotics resistance profiles of tested strains measured by I.Z.D* (mm) in MRSc agar medium after incubation at 37°C for 48 h.

Antibiotics / Strains	Rifampicin (30 µg)	Oxytetra-cycline (30 µg)	Amikacin (30 µg)	Norfloxacin (10 µg)	Cefadroxil (30 µg)	Cefoperazone (75 µg)
B. adolescentis a-1	42 ± 0.05 ^{AB}	20± 0.11 ^{A}	ND	ND	ND	ND
B. longum a-2	36 ±0.1 ^{DE}	12 ± 0.1 ^{C}	ND	ND	ND	ND
B. breve 116	42 ±0.05 ^{AB}	14 ± 0.05 ^{BC}	ND	ND	ND	ND
B. longum BB-536	44 ±0.1 ^{A}	16 ± 0.15 ^{B}	ND	ND	ND	ND
B. longum b-1	42 ± 0.11 ^{AB}	22 ± 0.11 ^{A}	ND	ND	ND	ND
B. bifidum Bb 12	40±0.05 ^{BC}	14 ± 0.15 ^{BC}	ND	ND	ND	ND
B. breve 1695	38 ±0.05 ^{CD}	16 ± 0.1 ^{B}	ND	ND	ND	ND
B. bifidum B3	34 ±0.1 ^{E}	ND	ND	ND	ND	ND
L. mesenteroides B-512f	36 ±0.05 ^{DE}	14 ± 0.05 ^{BC}	ND	ND	ND	ND
L. cremoris a-22	36 ± 0.15 ^{DE}	12 ± 0.15 ^{C}	ND	ND	ND	ND

Mean ± SE* ND = not detected I.Z.D* = inhibition zone diameter

* Means in each column followed with the same letter are not significantly different at 0.05 probability level.

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According to Table (2), all tested strains exhibited high resistance to some of the antibiotics used in this study namely (Amikacin, Norfloxacin, Cefadroxil and Cefoperazone). The other antibiotics, Rifampicin and Oxytetracycline inhibited most tested strains but Rifampicin was stronger than Oxytetracycline however, the inhibition strength of whether differed from strain to other. For instance, *B. Bifidum* B3 was susceptible to Rifampicin (inhibition zone diameter, I.Z.D. = 34 mm) while resisted Oxytetracycline (I.Z.D. = 0 mm). *B. longum* BB-536 had the highest sensitivity to Rifampicin (I.Z.D. = 44 mm) while obtained the third order in resistance to Oxytetracycline (I.Z.D. = 16 mm) compared to the other strains. With respect to Leuconostoc strains, *Leu. mesenteroides subsp. cremoris* a-22 had the lowest sensitivity to Oxytetracycline (I.Z.D. = 12 mm) followed by *Leu. mesenteroides subsp. mesenteroides* B-512f (I.Z.D. = 14 mm) and they were similar in Rifampicin resistance (I.Z.D. = 36 mm) compared to the other strains.

These results are partly in agreement with those obtained by Delgado et al., (2005) and D'Aimmo et al., (2007).

According to the foregoing results, *B. adolescentis* a-1, *B. longum* BB-536, *B. longum* b-1, *B. bifidum* Bb 12 and *L. mesenteroides* B-512f were the best strains among all tested strains whereas showed an excellent survival at all in vitro experiments performed. Therefore, these strains have been chosen for further study with some prebiotics.

The effect of some prebiotics on growth rate of selected probiotic strains

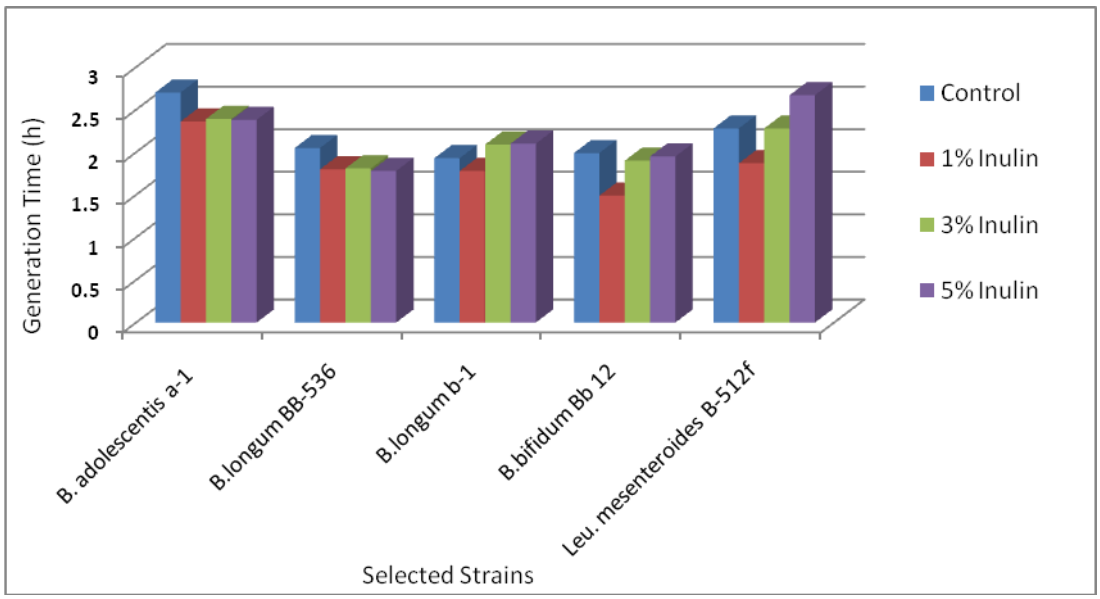

Fig (8): generation time (t_g) of selected strains with different concentrations of Inulin as prebiotic.

Important information about physiology of bacterial strains can be obtained by the study of the influence of culture conditions upon growth kinetics. The generation time was proposed as a tool to investigate the microbial dynamics either in pure or mixed cultures, and in this way the stimulating effect of inulin as prebiotic, on the growth of Bifidobacteria was confirmed (Bruno *et al.,* 2002). Therefore, the reduction of generation time of selected probiotic strains was calculated as evidence of the efficacy of prebiotic added to growth medium at different concentrations. Figs (8, 9 and 10) shows generation time (t_g) of each strain grown in MRSc broth supplemented with Inulin, Fructooligosaccharide (FOS) and Lactulose separately at concentration 0, 1, 3 and 5% (w/v). Inulin was suitable stimulant for all strains, for *B. longum* BB-536 at 5% Inulin t_g reduced from 2.05 to 1.78 hr while, it was suitable for *B. longum* b-1 (t_g = 1.78 hr), *B. bifidum* Bb 12 (t_g = 1.49 hr) and *Leu. mesenteroides subsp. mesenteroides* B-512f (t_g = 1.87 hr) at 1% Inulin (Fig. 8). FOS 1% was suitable concentration for all strains except *Leu. mesenteroides subsp. mesenteroides* B-512f which was stimulated at 5% FOS and its t_g decreased from 1.75 to 1.42 hr (Fig. 9). Lactulose was dispiriting of *B. bifidum* Bb 12 at all concentrations. For instance, 5% Lactulose was stimulant for *B. adolescentis* a-1 t_g decreased from 2.01 to 1.53 hr and *Leu. mesenteroides subsp. mesenteroides* B-512f stimulated with 1%

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Lactulose and its t_g decreased from 1.78 to 1.60 hr (Fig. 10). Generally, Inulin 1% was the best for *B. bifidum* Bb 12 (t_g = 1.49 hr), while FOS 5% was the best for *Leu. mesenteroides subsp. mesenteroides* B-512f (t_g = 1.42 hr) also, Lactulose 5% was the best for *B. adolescentis* a-1 (t_g = 1.53 hr). These findings are in agreement with those obtained by (Oliveira *et al.,* 2011) who found that Inulin supplementation as a prebiotic to improve quality of skim milk fermented by pure cultures of *Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus bulgaricus and Bifidobacterium lactis* lowered the generation time (tg) significantly ($p < 0.05$) of these strains as followed percents: 10.94, 9.09, 37.11 and 8.93%, respectively.

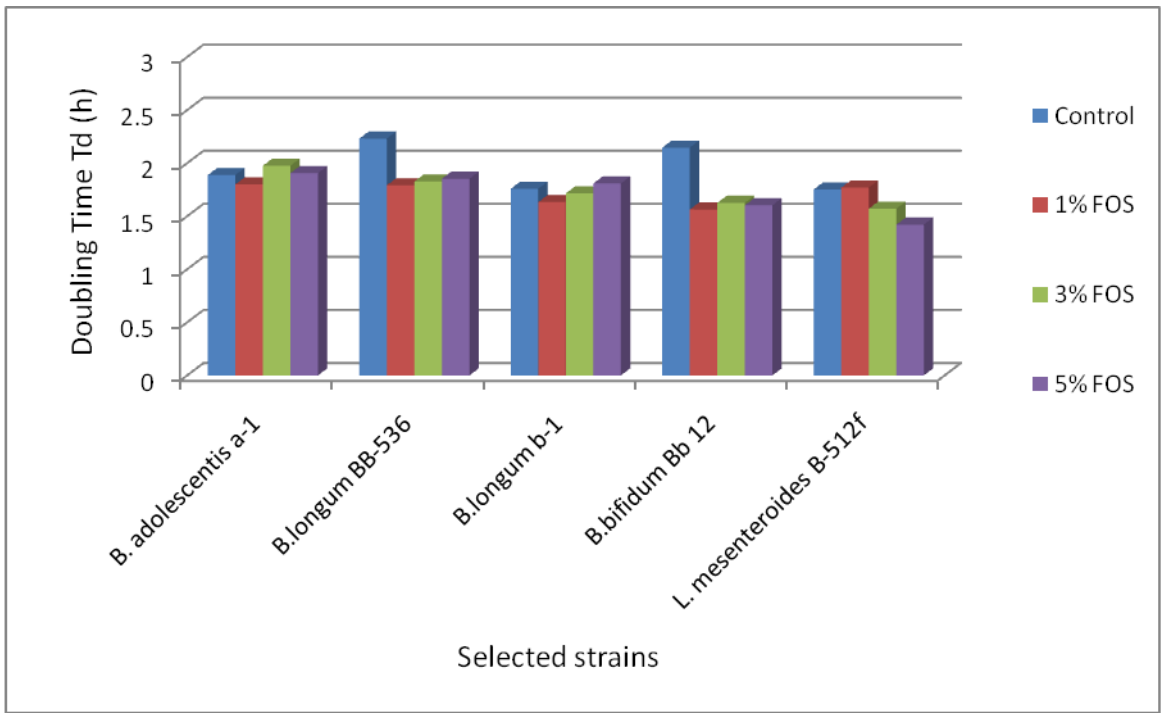

Fig (9): generation time (t_g) of selected strains with different concentrations of Fructooligosaccharide (FOS) as prebiotic.

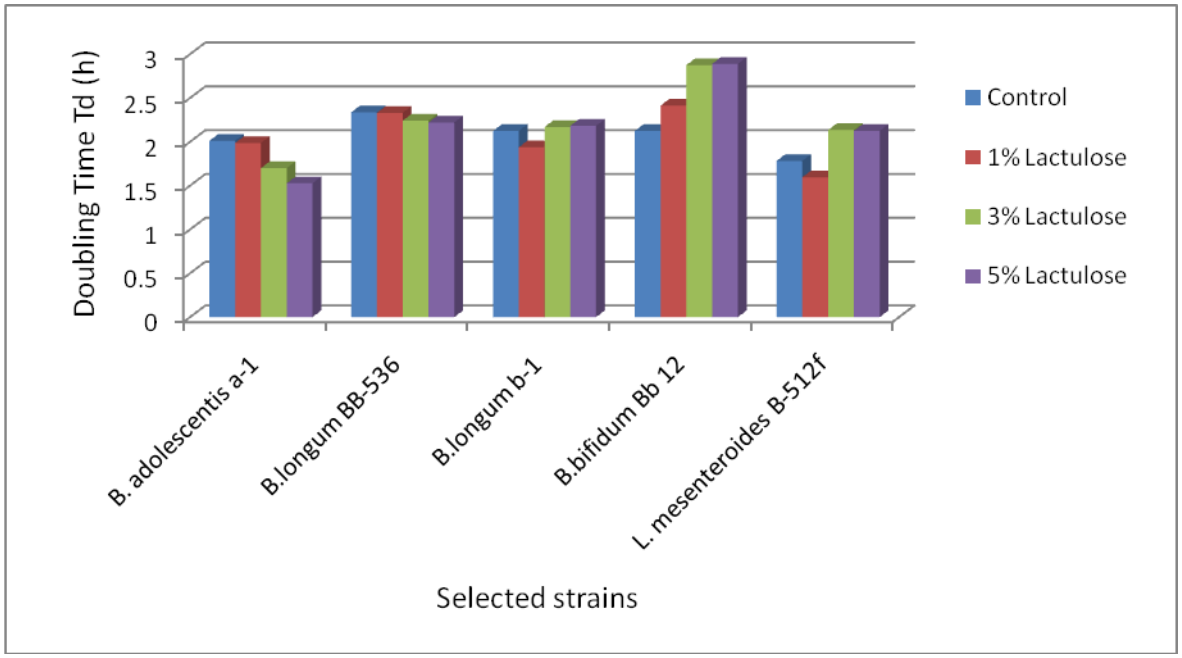

Fig (10): generation time (t_g) of selected strains with different concentrations of Lactulose as prebiotic.

In conclusion, *B. adolescentis* a-1, *B. longum* BB-536, *B. longum* b-1, *B. bifidum* Bb 12 and *Leu. mesenteroides subsp. mesenteroides* B- 512f strains showed a good survival at all *in vitro* tests performed.

Thus, these strains would have a high chance to survive humane digestive tract conditions and could be selected for use with with Inulin or Fructooligosaccharide as prebiotics in the functional foods.

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