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TOLERANCE OF PROBIOTIC BACTERIA TO ADVERSE CONDITIONS AND THEIR ADHESION TO INTESTINAL EPITHELIAL CELLS

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ABSTRACT

Dairy products is one of the most common carrier which have been used as probiotic food products. Many factors may affect the growth and survival of probiotic bacteria while it transit in dairy products to human use. To study these factors, bacterial strains belonging to three genera (*Bifidobactrium*, Streptococcus and *Lactobacillus*), in addition to two local isolates (Wh1 and Wh5) isolated from a whey sample were subjected to pH 4 ,bile salts 0.3% ,pH 3 and NaCl 7.5 %. They were also tested for attachment to sheep intestinal epithelial cells. The results indicated that strains of *Bifidobacterium angulatum* (2238) and *Bifidobactrium bifidum (LMG 10645)* and the local isolate Wh5 showed high tolerance to acidity, bile salts, sodium chloride and good attachment to sheep intestinal epithelial cells. Therefore, these bacteria are highly recommended to be used in the manufacture of bio-yoghurt as a functional food.

Key words: Probiotic bacteria, bile salt, acid tolerance, adherence.

INTRODUCTION

In the late nineteenth century, microbiologists identified microflora in the gastrointestinal tract of healthy animals that differed from those found in sick animals. As further research continued into the isolation and characterization of these microorganisms, it was revealed that ingestion of these bacteria could confer a wide range of therapeutic benefits to humans. These beneficial microflora were termed probiotics (Kotilainen *et al*., 2006).

Food and Agriculture Organization/World Health Organization Working Group (FAO/WHO, 2002) recognid probiotics as live microorganisms which when administered in adequate amounts confer a health benefit of the host. But the Joint International

Scientific Association for Probiotics and Prebiotics recently adopted this definition (Reid *et al*., 2003) ―Probiotic bacteria are live food supplements which benefit the health of the consumer.

The most commonly studied probiotic bacteria include members of the genus *Lactobacillu*s, especially, *L. acidophilus* and *Bifidobacterium* spp. (Tannock, 2002). These bacteria were found to prevent diarrhea in children, suppress pathogens in the intestinal tract, alleviate symptoms of lactose indigestion and enhance the population of beneficial bacteria in the human gut (Rafter, 2004; Sanders, 2000 and 2003).

The numerous perceived health benefits and the growing awareness about probiotics have caught the attention of the food industry (Saarela *et al*., 2002; Salminen and Gueimonde, 2004). Food companies are increasingly manufacturing foods with incorporated probiotic bacteria, which fall under the new category of foods called functional foods.

The consumption of probiotic bacteria within food products is the most popular way to re-establish the gastrointestinal microflora balance. The literature had stated that probiotic products have to present not less of 10^6 cfu /ml of probiotic bacteria at the time of consumption to get the beneficial health on the host (Lourens-Hattingh and Viljoen, 2001; Adhikari *et al*., 2003).

Dairy products is one of the most common carrier that have been used as probiotic food products **(**Lourens-Hattingh and Viljoen, 2001; TianHong and XiangChen, 2004). Therefore, it is of interest to study some factors that affect the growth and survival of probiotic bacteria while it transit in dairy products to human use.

Many factors have been reported to affect the growth and survival of probiotic bacteria in dairy products, including acid and hydrogen peroxide produced by yogurt bacteria, oxygen content in the product and oxygen permeation through the package and the storage temperature (Talwalkar and Kailasapathy, 2004; Bolduc *et al*., 2006). The survivability and viability of probiotic bacteria were one of the most interested elements in the probiotic world studies (Gilliland *et al*., 2002).

This work aimed to study the tolerance of some probiotic bacteria to certain adverse conditions (*i.e.* high acidity, bile salts and NaCl). Moreover, the adhesion of the probiotic bacteria to columnar epithelial cells of the small intestine of sheep was also studied.

MATERIALS AND METHODS

Probiotic bacteria:

Different probiotic bacterial strains belonging to three genera were kindly supplied by Cairo Microbiological Resources Center (MIRCEN) Faculty of Agriculture, Ain Shams University. These strains were as follow:

1. *Bifidobacterium angulatum* (2238), *Bifidobacterium bifidum* (2203), *Bifidobacterium animalis subsp .lactis* (BB12) and

Bifidobacterium bifidum (LMG 10645). These strains were encoded B1, B2, B3 and B4, respectively.

2. *Lactobacillus delbureckii ssp bulgaricus (EMCC 11102), Lactobacillus acidophilus*. These strains were encoded L1 and L2, respectively
3. *Streptococcus salivarius* subsp. *thermophilus* (EMCC 11044) was encoded (St). In addition, two isolates of lactic acid bacteria were isolated from whey sample and designated as Wh1 and wh5.

Isolation of bacteria:

The Standard method of the examination of dairy products (1978) was used to isolate probiotic bacteria using MRS medium **(De Man *et al*., 1960).** One gram of whey sample was serially diluted with 9 ml of 0.1 % peptone water. Appropriate dilutions were plated on MRS agar, and incubated anaerobically at 37˚ C for 72 hrs. Single colonies were isolated.

Tolerance of probiotic bacteria to acidic condition

Test tubes each containing 10 ml of MRS broth medium adjusted at pH 4 and pH 3 were prepared. After sterilization, the test tubes were inoculated with 1% v/v fresh liquid cultures of the probiotic bacteria (in three replicates). The bacterial growth was determined by measuring the optical density (660nm) at zero, after 24 and 48 hrs of incubation at 37ºC. The increasing rate of the growth was calculated according to the following equation:

Growth increase (Gl) = (A – B) /B

where: A = optical density (O.D.660nm) after 24 or 48hrs

B = optical density (660nm) at 0.0 time.

Tolerance of probiotic bacteria to bile salt

Growth of probiotic bacteria in MRS broth medium with added bile salts (Oxgall Sigma Chemical Co., St. Lois, Mo., USA) according to Gilliliand *et.al* (1984) was tested. Test tubes each containing 10 ml of MRS broth containing 0.3% bile salts were prepared. Tubes were inoculated with 1% v/v liquid cultures of the tested probiotic bacteria. Three replicates for each bacterium were involved. The growth was measured as optical density just after inoculation (zero time) and after 24 and 48 hrs of incubation at 37ºC. The increasing rate of the growth was estimated according to the equation mentioned above.

Bacterial adhesion to the intestinal epithelial cells:

Adhesion of bifidobacteria to intestinal columnar epithelial cells of sheep was tested using the procedure of Fuller (1973). The tested cultures (each in 10 ml MRS broth) were centrifuged and the pellets were resuspended in 10 ml of buffer saline (pH 7.2). The crop scrapings were prepared by removing the organ, opening with scissors and washing in buffer. The epithelium was then scraped off with the edge of a microscope slide and the scrapings were suspended in the buffer. 0.1 ml of bacterial cell suspension was added to 0.4 ml of the epithelial cell suspension. The mixture was rotated at 16 rev/min for 30 min at 37°C. Adhesion was examined and photographed by light microscopy of Gram stained preparations.

Tolerance of probiotic bacteria to NaCl

For the determination of NaCl tolerance of tested bacteria, test tubes containing MRS broth supplemented with 7.5 % of NaCl were prepared. After sterilization , the test tubes were inoculated with (1% v/v) fresh cultures of the tested bacteria (in three replicates) and incubated at 37ºC. Bacterial growth was determined by measuring the optical density at 660 nm just after inoculation (zero time) as well as after 24 and 48 hrs. The increasing rate of the growth was estimated according to the equation mentioned above.

RESULTS AND DISSECTION

Selection of the most tolerant Probiotic bacteria to acidity

Data presented in Table (1) indicate that all probiotic bacteria (9 isolates) exhibited tolerance to acidity (pH 4) expressed as optical density at 660nm and growth increase (GI). All tested isolates exhibited tolerance to acidic conditions, but tolerance varied from isolate to another. Among the nine probiotic isolates tested isolates L1 and B1 exhibited the highest growth (2.134 and 1.414, respectively) expressed as optical density after 24 hrs. In addition, the highest values of growth increase after 24 hrs. were recorded for L1 and wh5 which was found to be 3.378 and 3.139 folds, respectively.

Table (1): Tolerance of probiotic bacteria to acidic conditions (pH 4).

Source Isolate designation	Optical density at 660nm after (hr)				
	Zero Time	24 Hrs	Rate of increase (fold)	48 Hrs	Rate of increase (fold)
B1	0.364	1.414	2.881	1.594	3.376
B2	0.333	1.112	2.340	1.607	3.825
B3	0.340	1.197	2.519	1.754	4.155
B4	0.278	1.091	2.926	1.614	4.808
L1	0.487	2.134	3.378	2.215	3.545
ST	0.252	0.332	0.318	1.073	3.257
L2	0.222	0.416	0.873	1.401	5.310
Wh1	0.316	0.816	1.579	1.604	4.070
Wh5	0.313	1.297	3.139	1.857	4.927

Moreover, after incubation for 48 hrs. The highest growth values as optical density were found to be 2.215 and 1.857 for isolates L1 and wh5, respectively. Moreover, the highest growth increase values were 5.310 folds and 4.927 folds for isolates L2 and wh5, respectively.

It is well known that probiotic bacteria should be tolerant or resistant to gastric acid (HCL) for at least 90 minutes (Chou and Weimer, 1999). The obtained data in this study revealed that several isolates tolerated greatly pH 4 for as long as 48 hrs .In previous studies , several reports confirmed the good tolerance of probiotic isolates mainly lactobacilli to low pH levels (Rashid *et al*., 2007;Ashraf *et al* ., 2009 and Bao *et al*., 2009). Recently, Boke *et al*., (2010) explained the resistance to low pH to be due to the exopolysaccharides production by probiotics. They added that the high EPSS producing strains showed a significant protective effect against low pH (2.0).

Tolerance of Probiotic bacteria to Bile salts.

Microflora in the gastrointestinal tract of healthy animals are exposing to bile salts, therefore, bile tolerance is a desirable character for probiotic bacteria. Tolerance of the nine probiotic isolates under study to bile salts (at concentration of 0.3%) was tested.

As presented in Table (2) all probiotic isolates tested exhibited tolerance to bile salts (0.3%) even after 48 hrs. Values of growth expressed as optical density and growth increase varied from isolate to another. After 24 hrs the highest growth values were found to be 2.458 and 1.599 recorded for isolates L1 and B1, respectively. Whereas, the highest growth increase values were 4.946 folds and 4.069 folds for isolates L1 and B2, respectively. Moreover, after 48 hrs. the highest growth values (optical density) were found to be 2.905 and 2.623 for isolates L1 and wh5, respectively. Whereas, the highest growth increase values were found to be 8.584 folds and 6.572 folds for isolates wh5 and B2, respectively.

Tahri *et al.* (1995**),** reported that Gram positive bacteria were capable of hydrolyzing the amide bond of conjugated bile salts, liberating free bile salts with lower detergent properties. Bifidobacteria were found to excrete bile salt hydrolase's (BSH; cholylglycine hydrolase's), the enzyme that catalyses the hydrolysis of glycine and or taurine conjugated bile salts into amino acid residues and free bile salts. BSH was found to be present in several bacterial strain of the gastrointestinal tract, such as *B. longum* (Gorzo and Cilliland, 1999**).** The function of this enzyme in the producing bacteria is the utilization of the liberated amino acid and increase the resistance to the toxic levels of bile salts in the gastrointestinal environment (De Semet *et al*., 1995). Since, the tested isolates of probiotic bacteria exhibited high tolerance to acidic conditions (pH 4) and bile salts (0.3%), it was of a particular interest to study the stability of these isolates in higher acidity conditions (pH 3).

Data presented in Table (3) indicated that all of the tested bacteria were able to survive at pH 3. The bacterial isolates from whey (Wh5) showed high tolerance to pH 3 and growth increase (GI) was found to be 1.6 folds after 48 hrs. These results are in agreement with those obtained by AL-Awwad *et al*. (2009) and Hoque *et al* .(2010) who reported that lactobacilli and many probiotics were tolerant to pH 3 or lower.

Table (2): Tolerance of probiotic bacteria to 0.3% bile salt.

Isolate designation	Mean optical density at 660_{nm} after (hr)				
	Zero Time	24 Hrs	Rate of increase (fold)	48 Hrs	Rate of increase (fold)
B1	0.392	1.599	3.080	2.204	4.622
B2	0.293	1.488	4.069	2.223	6.572
B3	0.402	1.560	2.874	2.241	4.567
B4	0.378	1.584	3.183	2.255	4.955
L1	0.413	2.458	4.946	2.905	6.029
ST	0.324	0.346	0.066	1.785	4.497
L2	0.350	1.468	3.194	1.439	3.111
Wh1	0.410	0.445	0.085	2.255	4.500
Wh5	0.273	0.290	0.060	2.623	8.584

Table (3): Tolerance of Probiotic bacterial to high acidity (pH 3).

Source Isolate designation	Mean optical density at 660_{nm} after (hr)				
	Zero Time	24 Hrs	Rate of increase (fold)	48 Hrs	Rate of increase (fold)
B1	0.293	0.455	0.551	0.453	0.546
B2	0.279	0.383	0.373	0.364	0.304
B3	0.249	0.352	0.413	0.505	1.030
B4	0.254	0.486	0.910	0.441	0.732
L1	0.332	0.336	0.013	0.576	0.735
ST	0.224	0.414	0.848	0.289	0.293
L2	0.219	0.382	0.747	0.388	0.773
Wh1	0.289	0.376	0.301	0.550	0.903
Wh5	0.296	0.343	0.162	0.487	1.649

Adhesion of probiotic bacteria to sheep intestinal epithelial cells:

A major consideration in the choice of *Bifidobacterium* and other probiotic bacteria to be `used as dietary adjuncts must be the strain that cannot only survive in stomach acidity but also the establishment within the digestive tract. Therefore, the adhesion of bifidobacteria to columnar epithelial cells of the small intestine of sheep was tested. Fig. (1) shows the appearance of the sheep epithelial cells after the removal of the adherent bacteria. Moreover, adherence of the tested probiotic bacteria to sheep intestinal epithelial cells can be clearly seen. Good attachment of B1, B4 and Wh5 to epithelial cells was observed.

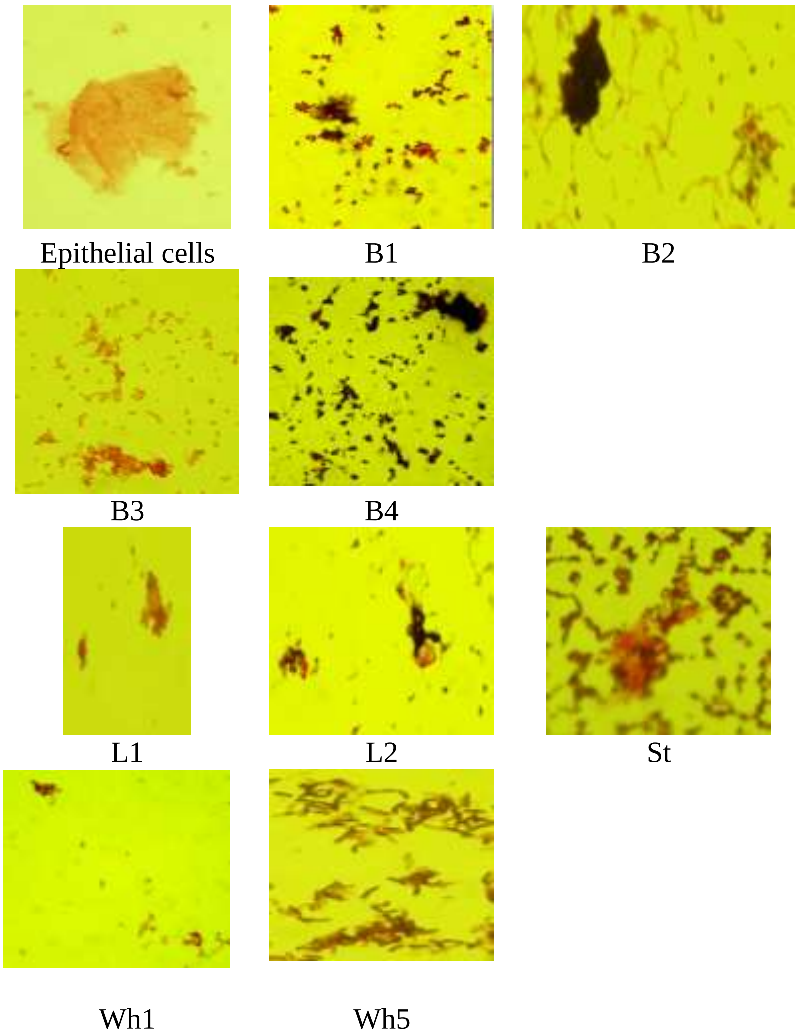

Fig. (1): Adhesion of probiotic bacteria to epithelial cells of small intestine of sheep.

Mayra Makinen *et al.,* (1983) demonstrated that adhesive bacteria showed a concentration of organisms on the epithelial cells. Gilliland *et al.,* (1975) reported some differences in the characteristics of organisms isolated from different hosts. The difference between *bifidobacterium* may be due to binding of the lipoteichoic acid of bifidobacteria to human colonic epithelial cells which appeared to be specific, reversible, and depend on the length of contact time and cell concentration (Fisher *et.al*., 1986). The higher the level of the fatty acid fractions of the lipoteichoic acids, the better the adhesion **(**OpDenkamp *et.al*, 1985)

Tolerance to NaCl(7.5%).

The tested bacteria were subjected to NaCl at 7.5%, as one of the characteristics of probiotic bacteria as reported by **(**Hoque *et al*., 2010).

The results in Table (4) showed that most of the isolates were tolerance at variable levels to NaCl at 7.5% for 48hrs . Among the nine tested isolates , three were found to be highly tolerant (B1 ,B4 and wh5) with rale of increase ranging from 6.03 to 6.28. Similarly**,** Erdourul and Erbilir (2006) reported that *L.bulgaricus* and *L.casei* were able to grow at 7.5% NaCl. in addition . Ruiz – Mayano *et al*. (2008) tested 1000 isolates for potential probiotic properties by their ability to grow adequately at different NaCl concentration. Hoque *et al*., (2010) found that *Lactobacillus* spp. isolated from yoghurt were able to tolerant 1-9% NaCl .

Table (4) Tolerance of tested bacteria to NaCl at 7.5%.

strain	Mean optical density at 660nm after (hrs)				
	Zero time	24 Hrs	Rate of increase (fold)	48 hrs	Rate of increase (fold)
B1	0.275	1.836	5.676364	2.003	6.283636
B2	0.301	1.692	4.621262	1.973	5.554817
B3	0.366	1.768	3.830601	1.997	4.456284
B4	0.278	1.715	5.169065	1.998	6.18705
L1	0.272	1.275	3.6875	1.406	4.169118
ST	0.285	1.074	2.768421	1.413	3.957895
L2	0.329	1.746	4.306991	2.001	5.082067
Wh1	0.334	1.102	2.299401	1.831	4.482036
Wh5	0.255	1.382	4.419608	1.793	6.031373

CONCLUSION

On the basis of the obtained results it can be concluded that, isolates B1, B4 and wh5 showed high tolerance to acid, bile salt, sodium chloride and good attachment to epithelial cells that encourage using them in the manufacture of bio-yoghurt as a functional food.

REFERENCES

Adhikari, K; Mustapha, A. and Grun, I.U. (2003): Survival and Metabolic Activity of Microencapsulated *Bifidobacterium longum*in Stirred Yogurt. Journal of Food Science;68: 275-280.

AL-Awwad, N.J. ; Haddadin,M.S. and Takruri, H.R.(2009):The characteristics of locally isolated lactobacillus acidophilus and Bifidobacterium infantis isolates as probiotics Strains . Jordan J.Agric. Sci., 5(2):192-206.

Ashraf,M.; Arshad,M.; Siddique,M.and Muhammad,G.(2009) : In Vitro screening of locally isolated of *lactobacillusspecies* for probiotic .properities .Pakistan Vet.J., 29(4):186-190.

Bao,Y.;Zhang,Ya.; Zhang,Yo.;Liu ,Y.; Wanga ,S. ; Dong,X.; Wang,Y.and Zhang,H. (2009): Screening of potential probiotic properties of *lactobacillusfermentum* isolated from traditional dairy products .J.FoodCont, 21:695-701.

Boke,H.; Aslim, B.and Alp,G.(2010) : The role of resistance to bile salts and acid tolerance of exopolysaccharides (EPSS)produced by yoghurt starter bacteria . Arch .Biol.Sci.,Belgrade , 62(2),323-328.

Bolduc, M.P.; Raymond, Y.; Fustier, P.; Champagne, C.P. and Vuillemard, J.C. (2006). Sensitivity of bifidobacteria to oxygen and *Journal;* 16*:* 1038-1048.

Chou,L.S .and Weimer,B. (1999):Isolation and characterization of acid – and bile – tolerant isolates from strains of *lactobacillusacidophilus*. J. Dairy Sci. 82(1):23-31.

De Man, J.C. Rogosa, M., Sharpe, Elisabeth (1960) "A Medium for the Cultivation of Lactobacilli". J. Appl. Bact. 23. 130-135.

De Semet, H.V.; Hoorde, M.V.; Woestyne, H.; Christiaens, W. and Verstraete, E. (1995). Significance of bile salt hydrolase's activity of Lactobacillus. J Applied Bacteriology. 79: pp. 292-301.

Erdourul, O. and Erbilir,F. (2006) : Isolation and characterization of lactobacillus bulgaricus and lactobacillus casei from various foods .Turk J. Biol., 30:39-44.

FAO/WHO. (2002). Guidelines for the Evaluation of Probiotics in Food. London Ontario, Canada.

Fisher, R.R.; Glatz, C.E. and Murphy, P.A. (1986).Effects of mixing during acid addition on fractionally precipitated protein Biotechnology and Bioengineering. 28(7): 1056-1063.

Fuller, R. (1973): Ecological on the Lactobacillus flora associated with the Grope epithelium of the fowl. J. Appl. Bactl. 36: 131-139.

Gilliland, S.E.; Spech, M.L. and Morgan C.G. (1975) Appl. Microb. 30: 541-545.

Gilliland, S.E.; Nelson, G.R. and Maxwell, C. (1984): Assimilation of cholesterol by *Lactobacillus acidophilus*. Applied Environ Microb. 49: 377-385.

Gilliland, S.E.; Reilly, S.S.; Kim, G.B.; and Kim, H.S. (2002). Viability During Storage of Selected Probiotic Lactobacilli and Bifidobacteria in a Yogurt-like Product. *Journal of Food Science*; 67: 3091-3095.

Gorzo, G. and Cilliland, S.E. (1999). Bile salt hydrolase activity of three strains of *Lactobacillusacidophilus*. J. Dairy Sci. 82: 472-480.

Hoque, M.Z.;Akter,F.; Hossain,K.M.; Rahman,M.S.M;Billah, M.M. and Islam ,K.M.D.(2010) :Isolation , identification and analysis of probiotic properties of *lactobacillus spp*. From selective regional yoghurts .world J. Dairy & Food Sci. 5 (1) : 39-46.

Kotilainen, L.;Rajalahti, R.;Ragasa, C. and Pehu, E. (2006). Health Enhancing Foods Opportunities for Strengthening the Sector in Developing Countries. Agriculture and Rural Development Discussion Paper 30.

Lourens-Hattingh, A. and Viljoen, B.C. (2001). Yogurt as probiotic carrier food. *International Dairy Journ al;* 11: 1-17.

Mayra-Makinen, A.; Manninen, M. and Gyllenbery, H. (1983). The adhesion of lactic acid bacteria to the columnar epithelial cells of pigs and caves. J Appl Bact. 55: 241-245.

Op Denkamp, H.J.M.; Peeters, P.A.M.; Oosterhof, A.; Veerkam, J.H.J. (1985). J. Gen Micro. 131: 661-668.

Rafter, J. (2004). The effects of probiotics on colon cancer development. *Nutrition Research Reviews*; 17: 277-284.

Rashid,M.H; Togo,K.;Ueda,M.and Miyamoto, T. (2007): Probiotic characteristics of lactic acid bacteria isolated from traditional fermented milk Dahi in Bangladesh .Pakistan J. Nutrition 6 (6): 647-652.

Reid, G.; Sanders, M.E.; Gaskins, H.R.; Gibson, G.R.; Mercenier, A. ;Rastall, R.;Roberfroid, M.;Rowland, I.;Cherbut, C. and Klaenhammer, T.R. (2003). New scientific paradigms for probiotics and prebiotics. *Journal of Clinical Gastroenterology*; 37: 105–118.

Ruiz-mayano, S. ;Martin, A. ;Benito, M. J.; Nevado, F. P. and Cordoba, M. G. (2008): Screening of lactic acid bacteria and bifidobacteria for potential probiotic use in lberian dry fermented sausages . Meat Sci.,80(3): 715-721.

Saarela, M.;Lahteenmaki, L.;Crittended, R.;Salminen, S. and Mattila-Sandholm, T. (2002). Gut bacteria and health foods-the European perspective. *International Journal of Food Microbiology*; 78, 99-117.

Salminen, S.; and Gueimonde, M. (2004). Human Studies on Probiotics: What is Scientifically Proven.*Journal of Food Science*; 69: M137-M140.

Sanders, M.E. (2000). Symposium: Probiotic Bacteria: Implications for Human Health - Considerations for use of probiotic bacteria to modulate human health. *American Society for Nutritional Sciences;* 130: 384S-390S.

Sanders, M. E. (2003). Probiotics: Considerations for human health. *Nutrition Reviews*; 61: 91- 99.

Tahri, K.; Crociani, J.; Ballongue, J. and Schneider, F. (1995). Effects of three strains of bifidobacteria on cholesterol. Letters of ApplMicrob. 21: 149-151.

Talwalkar, A. and Kailasapathy, K. (2004). A Review of Oxygen Toxicity in Probiotic Yogurts: Influence on the survival of probiotic bacteria and protective techniques. *Comprehensive reviews in Food Science and Food Safety*; 3: 117-124.

Tannock, G.W. (2002). The Bifidobacteria and Lactobacillus microflora of humans. *Clinical Reviews in Allergy and Immunology*; 22: 231-253.

TianHong, B. and XiangChen, M. (2004). Probiotic and Probiotic cultured dairy products. *China Dairy Industry*; 32: 32-34.

الملخص العربى

تحمل بكتيريا البروبيوتك للظروف غير الملائمة وقدرتها على الالتصاق بالخلايا الطلائية للامعاء

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تعتبر منتجات الالبان اهم حاملات البكتريا النشطة حيويا واكثرها شيوعا كمنتجات غذائية وظيفية . وتؤثر العديد من العوامل على نمو وحيوية هذة البكتريا خلال مرورها من المنتجات اللبنية و حتى استهلاكها.

ولدراسة هذة العوامل اختيرت 9 سلالات تنتمى لاجناس *Streptococcus, Bifidobacterium, Lactobacillus* ودرجة تحملها لــ pH 4 واملاح الصفراء بتركيز 3% وpH 3، كلوريدالصوديوم بتركيز % 7.5 واختبرت ايضا قدرتها على الالتصاق بالخلايا الطلائية للامعاء واظهرت النتائج ان ثلاثة سلالات لهما القدرة على النمو بمعدل عالى على pH 3 وكذلك تحملها لاملاح الصفراء ومقدرة على الالتصاق بالخلايا الطلائية و تحملها تركيز % 7.5 من كلوريد الصوديوم . ولهذا ينصح باستخدام هذه البكتيريا فى صناعة الزبادى كمنتجات غذائية وظيفية.