Journal of Home Economics

Volume 24, Number (1), 2014

Journal of Home Economics

http://homeEcon.menofia.edu.eg **ISSN 1110-2578**

Selection Of Some Lactobacilli And Bifidobacteriastrains As Potential Probiotic Cultures

KhaterK.A. A.

Dairy Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt

Abstract: The in vitro and in vivo roles of **Lb. acidophilus** ATCC 4356, **Lb.johnsonii** ATCC 33200 and **B. bifidum** ATCC 15696 were investigated to explore their potential to survive transit through gastrointestinal tract and to inhibit coliform. These three cultures were tested, in vitro, for tolerant gastric and intestinal juices, they exhibited acceptable levels of survivability ranged from 93.95% to 98.75% and from 84.07% to 94.48% ingastric and intestinal juices respectively.

Short-term administration (2h) of the three tested strains in chicks, in vivo, confirmed their efficacy to passage successfully through the gastrointestinal tract. Also, **B. bifidum** ATCC 15696 recovered from the small intestine showed the least decrease in count after 2h of feeding and ranked the highest survival %, being 90.41%.

Moreover, the tested cultures were also examined in coculture with**E. coli** O157:H7ATCC 51657 in skim milk medium for their antagonistic effect. Results obtained showed that **B. bifidum** ATCC 15696 exert strong inhibitory activity toward **E. coli**, followed by **Lb. acidophilus** ATCC 4356. This statement may be attributed to the production of antimicrobial agents.

In addition, feeding experiment had carried out, in vivo by using Fayomy chicks to evaluate the antagonistic activity of the tested cultures towards **E. coli** during their passage through the gastrointestinal tract. The coadministration of **E. coli** and **B. bifidum** ATCC 15696 resulted in the decrease in **E. coli** count in feces samples, being 58.75%, followed by **Lb. acidophilus** ATCC 4356, actually 57.22%.

Generally, the obtained results strongly suggest that the tested strains were considered resistant to gastrointestinal transit, and this observation coupled with their good gastric and intestinal juice tolerance. Continuously, these cultures were able to inhibit **E. coli** in both coculture and intestines of chicks. The effect are seen in a short

period (72h), suggesting that these cultures may have clinical application for people suffering from gastrointestinal disorders caused by coliforms.

Introduction

A more recent definition of term probiotic was given by David and Michael (2008) as " living microorganisms or component of microbial cells that have beneficial effects on host ". However, a number of health benefits have been claimed for probiotic bacteria such as ***Lactobacillus*** and ***Bifidobacterium spp***.

Indeed, probiotics have been reported to play therapeutic roles by promote good digestion, boost immune function, inhibit the growth of harmful bacteria, removal of carcinogens, lowering of cholesterol, immune-stimulating and allergy lowering effect (Patricia et al., 2002; Helland et al., 2004; Grajek et al., 2005 and Parvez et al., 2006). Therefore, these organisms are increasingly incorporated into dairy products.

However, these bacteria to be considered as probiotics, they should become a part of the normal flora in the intestine, survive the gastrointestinal passage and be able to adhere and colonize in the intestinal tract (Havenaa et al., 1992). The human gastrointestinal tract is a harsh environment because it contains gastric juices, digestive enzymes and bile acids. These conditions impose significant threat to probiotic strains.

Therefore, a stringent selection criteria for identification of probiotic strains is required in order to achieve consistent and positive probiotic effects. In 1998, Collins et al. have compiled a list of 12 important criteria for selecting a potential probiotic strain. Essentially, these criteria suggested that the selected strains must be able to survive through their passage in the gastrointestinal tract and had the ability to exert antagonistic activity toward some pathogenic and spoilage bacteria, such as ***Escherichia coli*** and ***Bacillus spp***.

Thus, it was the main objective of this study was to compare the in vitro evaluation of survival of the three tested probiotic cultures for gastric and intestinal juices, that are important for their survival in the gastrointestinal tract, with their actual ability to survive in vivo.

Also, an additional target of this investigation was to examine the antagonistic effect of the tested probiotic cultures against***E.coli*** either in vitro or in vivo.

Material And Methods:

Testedcultures:*Lb. acidophilus* ATCC 4356, ***Lb. johnsonii*** ATCC 33200 and ***B. bifidum*** ATCC 15696 were secured from American typ culture collection, Manassas, USA. Bacterial cultures were propagated

daily in 10% sterilized skim milk, maintained in M-17 broth (Terzaghi and Sandine, 1975) and stored at 4°C until used.

Escherichia coli O157: H7 ATCC 51657 was purchased from Botany and Microbiology Department, Faculty of Science, Al-Azhar University (Assiut branch).

A stock culture was maintained through bimonthly transfers on nutrient agar and kept at 4°C. In order to propagate the pathogen, two transfers were carried out into nutrient broth, followed by incubation at 37°C for 16 hours.

Birds (Fayomy chicks): six weeks old Fayomy chicks were obtained from Poultry experimental station, Faculty of Agriculture, Al-Azhar University, Cairo. These birds were acclimatized on basal diet for one week before starting the experiments.

Basal diet: this diet consisted of: ground yellow corn, 60.8%, soybean meal, 20.7%, corn gluten meal,10.8%, dicalcium phosphate, 1.17%, Limestone, 5.76%, premix. 0.3%, NaCl, 0.3% and L-Lysine HCL,0.17%.

In vitro tolerance to gastric and intestinal juices:gastric and pancreatic (intestinal) juices were prepared fresh by dissolving pepsin (sigma) from porcine stomach mucosa (3g/L) and sterile saline (5g/L) according to Charteris etal. (1998) subsequently, the pHs of gastric and pancreatic preparations were, respectively, adjusted to 2.0 and 8.0 with 12M HCL or 0.1M NaOH.

The tolerance of the three tested strains to gastric and intestinal juices was determined by mixing 0.2ml of each washed cell suspensions with 1.0ml of gastric (pH 2.0) or intestinal juices (pH 8.0). After brief vortexingthe mixtures were incubated at 37°C. When assaying gastric tolerance aliquots of 0.1ml were removed after 60,120 and 180 min for determination of total viable count. While for assaying small intestinal tolerance, the sampling times were 60,240 and 360 mine. The experiment was repeated twice.

In vivo the survival of tested cultures in gastrointestinal tract: At the end of adaptation period (7 days),the birds were starved for 18h, randomly and equally divided into three groups, three birds each.

Three grams of concentrated ***Lb. acidophilus*** strain were mixed with 3g of basal diet and gave to the birds in the first groups. In the same manner, the second and third groups were received basal diet plus 3g of eitherconcentrated***L. johnsonii***or***B. bifidum***, respectively. After 2hof feeding, the chicks were killed, and the levels of viable count of each tested strain in the small intestinal were quantified (Abou-Dawood,2002)

In vitro inhibition of *E. coli*in coculture:

In order to explore the potential of the three tested cultures in inhibiting ***E.coli***, the effect was tested in coculture with ***E. coli***, in skim milk medium.

Each of the active tested cultures was inoculated at 1% (vol/vol) to 10% sterile skim milk medium and incubated for 48h at 37°C. For coculture studies a 1% (vol/vol) of an active culture of ***E. coli***was incubated to sterile skim milk medium. Samples were taken at different intervals and viable counts were determined and initial and final pH of all samples was also measured (Sreekumar and Hosono,2000).

In vivo Antagonistic of probiotics against *E. coli*:

After an adaptation period (7 days) and starved time for 18h, twenty Fayomy chicks were randomly and equally divided into five groups, four birds each. The first group was offered basal diet (15g) plus 5g of concentrated ***E.coli*** culture (10^3cfu/g), and served as control.While, the other groups were fed on 10g basal diet +5g of concentrated ***E. coli***+5g of concentrated cells (10^6cfu/g) of one of the tested cultures.

The concentrated cells were mixed with basal diet and were fed each morning during the experimental period. After ensuring the complete consumption of cells (approximately 2 h.), additional portions of normal diet were given, water were offered and libitum for birds during the experimental period.

Fecal Microbial Analysis:

All fecal samples were collected daily by gently squeezing the rectal area of the bird. The fecal pellets were immediately placed in tubes ket in anaerobic jars and the analysis was carried out within 30 to 60 min of collection.

Each sample was homogenized and a series of 10-fold dilutions of the specimens was made in serial phosphate buffer solution. Triplicate plates were made of each sample in violet red bile agar (VRBA) suggested by Misra and Kuila (1994 b), plates were incubated at 37°C for 2 days (Sreekumar and Hosono, 2000).

Microbiological Analysis:

For selective enumeration of ***B. bifidum***, modified MRS (m-MRS) medium consisted of MRS medium supplemented with 0.5 g/L.L-cystein HCL and 3.0g/L. Lithium chloride, was used(Dave and Shah, 1996).

While, for enumeration of ***L. acidophilus***, MRS-salicin agar medium, where a salicin concentration of 0.5% was appropriate for producing proper size colonies. (Shah, 2000). In addition,***Lb. johnsonii*** was enumerated on MRS-agar (Man, de Rogosa-Sharp, 1960).

For bacterial count, the general plate count technique outlined in the Standard Methods for Examination of Dairy products (A.P.H.A, 1978) was adopted.

The plates were incubated in gas jars with BBL GasPak anaerobic system envelops at 40°C for 72 h.

Results And Discussion

In vitro gastrointestinal transit tolerance:

The gastrointestinal transit tolerance of the three tested culture (***Lb. acidophilus*** ATCC 4356, ***Lb. johnsonii*** ATCC33200 and ***B. bifidum*** ATCC 15696) was determined in vitro by exposing washed cell suspension to a simulated gastric juice (SGJ) (pH 2.0), containing pepsin (3g/L) and sodium chloride (5g/L), and to a simulated small intestinal juice (SIJ) (pH 8.0) containing pencreation (1g/L) and sodium chloride (5g/L), mimicking the gastrointestinal environment.

Results obtained for tolerant gastric juice are shown in Table (1) and Figure (1). From these results it could be noticed that gastric juice exerted noticeable influences on the growth of all tested cultures, while tolerance to gastric juice varied among the tested strains. In general, ***Lb.johnsonii*** ATCC33200 exhibited more gastric resistance, followed by ***Lb. acidophilus*** ATCC 4356, while, ***B. bifidum*** ATCC 15696 was the most sensitive culture to gastric juice. However, variation in the tolerance to gastric juice was previously reported by Mathara et al. (2008); Ahmed (2009) and Kershah (2014).

Also, it was of interest to notice that although a slight reduction was detected in the viable cell counts, varied from 0.10 to 0.48 log cycle, for all tested cultures after exposure to gastric juice for 60 min., which is the time required for stomach content to empty nearly completely, the three tested cultures exhibited acceptable levels of survivability, ranged from the 93.95% to 98.75%.

In addition, data presented in Table (1) and Figure (1) also revealed that viable cell counts and survival % for all tested cultures were gradually decreased by prolongation of exposure time to gastric juice up to 180 min., where , the viable counts varied from 6.00 to 6.77 cfu/ml, while, survival % ranged from 75.47% to 84.62%. In this respect, with regard to results recorded by Guerra et al. (2007), they considered ***Enterococcus faecium*** CECT 410, intrinsically tolerant strain to gastric juice, since at least 6.30 log cfu/g survived after 180 min of exposure. Thus, our tested cultures were actually considered intrinsically tolerant gastric juice.

Generally, our present results revealed that all tested cultures could be successfully transit the stomach, reaching the intestinal tract and functioning effectively there.

The effect of simulated intestinal juice on the viability of the three tested strains is shown in Table 2 and Fig. 2. From results obtained it could be observed that ***Lb. acidophilus*** ATCC 4356 was markedly better with regard to intestinal juice tolerance than other tested cultures followed by ***B. bifidum*** ATCC 15696. In this respect, Mitsuok (1992)

reported that ***Lb. acidophilus*** is most active in the small intestine and ***B. bifidum*** is most active in the large intestine of humans.

Additionally, from data obtained, it could be concluded that the three tested strains strongly considered intrinsically tolerant to intestinal juice. In this connection, Charteris et al. (1998) stated that the majority of probiotic strains were intrinsically resistant to simulated pancreatic juice and showed no reduction in viability up to 4 hours.

In vivo gastrointestinal transit tolerance:

In order to estimate the amount of ingested bacteria surviving, transit through the gastrointestinal tract, feeding experiment had been carried out in vivo by using Fayomychicks. Each of the three tested cultures was mixed separately (10^6- 10^7cfu/g) with 3g of basal diet and ingested in the tested chicks.

Data obtained from this experiment presented in Table (3) and plotted in Figure (3). From these results , it could be stated that after two hours of feeding the viable cell count of ***B. bifidum*** ATCC 15696 recovered from the small intestinal was reduced by only 0.70 log cycle, and ranked the highest survival %, actually 90.41% . In this connection, AbouDawood (2002) gave a lower figure for survival % of ***B. bifidum***, being 71.43% while, a close value of 81.30% was reported by Elhadidi (2014).

On the other hand the population ***Lb. acidophilus*** ATCC 4356 showed noticeable decrease, being 1.65 log cycle, where the viable ingested count reduced from 6.65 log cfu/g to 5.0 log cfu/g when recovered after 2h from the small intestine. Additionally it was evident from data obtained that ***Lb. acidophilus*** ATCC 4356 attained the highest value for growth inhibition %,
actually 24.81% as compared with other tested strains. The same finding was previously reported by Sultana et al. (2000), Kershah (2014) and Elhadidi (2014).

Continuously, similar trend of result was also detected in case of ***Lb.johnsonii***ATCC33200, where the viable cell count reduced by 1.47 log cycle, while the survival % attained 78.29%.

Generally, the previous results strongly confirmed the efficacy and sutability of the tested cultures to passage through gastrointestinal tract successfully.

In vitro antagonistic effect of tested cultures (coculture test):

Lb. acidophilus ATCC 4356, ***Lb. johnsonii*** ATCC 33200 and ***B. bifidum*** ATCC15696were tested for their antagonistic effect against ***E. coli*** O157:H7ATCC 51657. These three cultures were tested in coculture with ***E. coli*** in skim milk medium. The viable count and pH were measured.

In Table 4 & 5 and Figures 4 & 5, the growth pattern of ***E. coli*** shows that the stationary phase was reachedin 36 h, and pH dropped to 5.7. A 2.3 log cycles increase of cell count was observed in 36 hours.

Also, it was evident from data obtained in Table 4 and Fig. 4 that ***E. coli*** failed to grow in coculture with ***Lb. acidophilus*** ATCC 4356 and the number of viable count was approximately constant until 12 h., after which the viability decreased sharply by about 2.7 log cycles after 24 h. of incubation. This was probably caused by the acid production along with other inhibitory compound produced during the fermentation of milk by ***Lb. acidophilus*** strains. In this respect, Hosono et al. (1977) mentioned to the production of antibiotic-like substances by ***Lb. acidophilus*** IFO 3205, which could repress the growth of ***E. coli***. Moreover Oh et al. (2000) stated that ***Lb. acidophilus*** strains produce a heat-stable antimicrobial compound that was shown to be proteinaccous in nature and referred to as bacteriocin.

Furthermore, it was of interest to notice that ***B. bifidum*** ATCC 15696 found to exert strong inhibitory activity toward ***E. coli***, the count of the pathogen was sharply reduced throughout incubation time and attained the lowest figure, being 1.69 log cfu/g at the end of incubation time. This finding may be attributed to the production of antimicrobial agents e.g. lactic, acitcacids and bifidin (Wijsman et al., 1989; Kebary, 1995 and Badawi& El-Sonbaty, 1997). Also, the same conclusion was also recorded by Makras and De Vuyst (2006).

From the same results (Table 4), it could be seen that ***Lb. johnsonii*** ATCC 33200 possessed the lowest antagonistic effect against ***E. coli***, where the count of the tested pathogen reduced only from 4.60 log cfu/g to 2.47 log cfu/g after 48 h of incubation. However, the decrease in the viable count of ***E. coli*** was proportional to the increased growth of ***Lb. johnsonii*** ATCC 33200. The acid produced with the antimicrobial substance during growth may be the responsible for the decline in the ***E. coli*** count.

Also, it was evident from data obtained that ***E. coli*** ranked the lowest growth inhibition %, being 46.30% when grow in coculture with***Lb. johnsonii*** ATCC 33200, as compared with the corresponding values, actually 58.70% and 63.26% in case of ***Lb. acidophilus*** ATCC 4356 and***B. bifidum*** ATCC 15696, respectively.

Thus, this study confirms that coliforms can be inhibited in vitro, in coculture by ***Lb. acidophilus*** ATCC 4356, ***Lb. johnsonii*** ATCC 33200 and***B. bifidum*** ATCC 15696 strains.

In vivo antagonistic effect of tested cultures (Feeding experiment):

In order to evaluate the ability of the tested cultures to exert antagonistic activity towards ***E. coli*** O157:H7during their passage through the gastrointestinal tract, feeding experiment had been carried

out in vivo by using Fayomy chicks. Birds were divided and treated as described previously.

From results obtained (Table 6 and Figure 6), it could be noticed that, in control treatment has resulted, as expected, in increased number of ***E. coli*** in feces samples by about 2.5 log cycles and attained 7 log cfu/g after 3 days of feeding versus the 0-day count, being 4.5 log cfu/g.

The coadministration of ***E. coli*** and ***Lb. acidophilus*** ATCC 4356, carried out in the seconed group of birds. The coadministrationresulted in noticeable decrease in ***E. coli*** count in feces samples and 2.5 log cycle decrease was detected. This finding may be attributed to the production of antimicrobial agents by ***Lb. acidophilus*** ATCC 4356 which suppres the growth of ***E. coli*** (Gibson and Wang, 1994 and Kebary, 1995). Thus, this result confirm that the consumption of viable cells of ***Lb. acidophilus*** ATCC 4356 may be able to control the gastrointestinal disorders caused by ***E. coli***. The same conclusion was previously reported by Sreekumar and Hosono (2000).

In contrast, ***Lb. johnsonii*** ATCC 33200 showed only minor inhibition effect against ***E. coli*** and possessed the lowest Ginh %, being 41%, as compared with those of ***Lb. acidophilus*** ATCC 4356 and ***B. bifidum*** ATCC 15696, being 57.22% and 58.75% respectively.

Continuously, the present results declared that the highest reduction in ***E. coli*** count in feces samples, being 58.75% was obtained when coadministration of ***E. coli*** and ***B. bifidum*** ATCC 15696 was adopted for 3 days, where the counts reduced by 2.35 log cycles. This statement may be ascribed to antagonistic effect of ***B. bifidum*** strain (Vijayendra& Gupta, 1992 and Kebary et al., 1996).

Generally, from the foregoing results, it could be pointed out that the three tested organisms can inhibit ***E. coli*** in vitro, in coculture and in vivo in the intestines of chicks. Also, the consumption of these strains as viable cells may result in controlling the intestinal flora especially for people suffering from gastrointestinal disordes caused by coliform. Further studies need to be carried out in human subjects to confirm this view.

Table (1) Effect of simulated gastric juice on viability of tested strains.

	Incubation time (min)						
	Zero	60		120		180	
	Log cfu/ml	Log cfu/ml	G. inh. (%)	Log cfu/ml	G. inh (%)	Log cfu/ml	G. inh (%)
L.acidophilus ATCC 4356	7.77	7.30	6.05	6.95	10.55	6.17	20.60
L.johnsonii ATCC33200	8.00	7.90	1.25	7.00	12.50	6.77	15.38
B.bifidum ATCC15696	7.95	7.47	6.04	6.84	13.96	6.00	24.53

Cfu/ml = Colony forming unit / ml
G. inh (%) = Growth inhibition percent

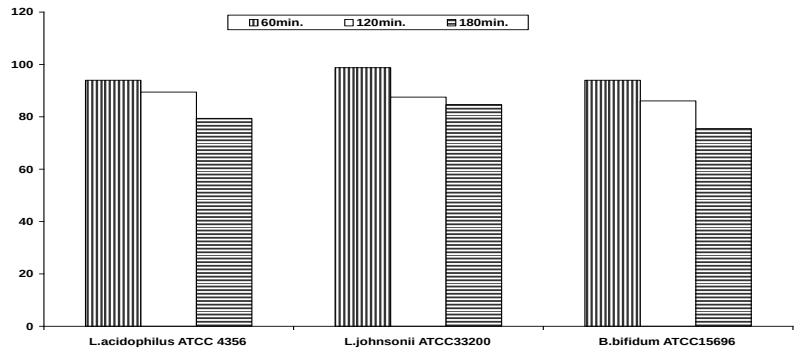


Fig. (1): Survival (%) of tested cultures at simulated gastric juice.

Table (2) Effect of simulated intestinal juice on viability of tested strains.

Tested strains	Incubation time (min)						
	Zero	60		240		360	
	Log cfu/ml	Log cfu/ml	G. inh (%)	Log cfu/ml	G. inh (%)	Log cfu/ml	G. inh (%)
L.acidophilus ATCC 4356	7.95	7.48	5.91	7.30	8.18	6.90	13.21
L.johnsonii ATCC33200	9.04	8.70	3.76	8.30	8.19	7.60	15.93
B.bifidum ATCC15696	8.15	7.70	5.52	7.48	8.22	6.95	14.72

Cfu/ml = Colony forming unit / ml
G. inh (%) = Growth inhibition percent

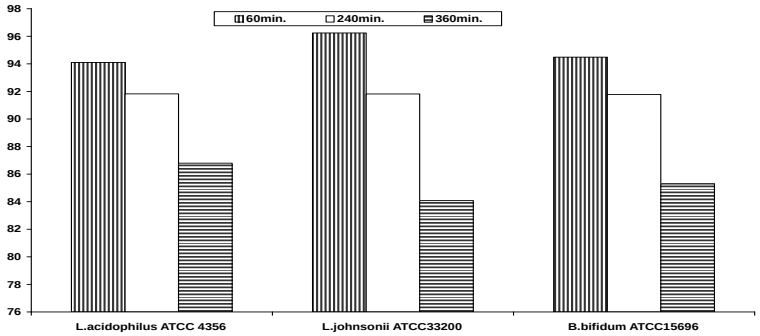


Fig. (2): Survival (%) of tested cultures at simulated intestinal juice.

Table (3) Level of administered and recovered of three tested cultures to chicks.

Tested strains	Digestion time (min)			
	Zero	120	G. inh (%)	Survival (%)
	Log cfu/ml	Log cfu/ml		
L.acidophilus ATCC 4356	6.65	5.00	24.81	75.19
L.johnsonii ATCC33200	6.77	5.30	21.71	78.29
B.bifidum ATCC15696	7.30	6.60	9.59	90.41

CFu/g = Colony forming inhibition percent
G. inh (%) = Growth inhibition percent

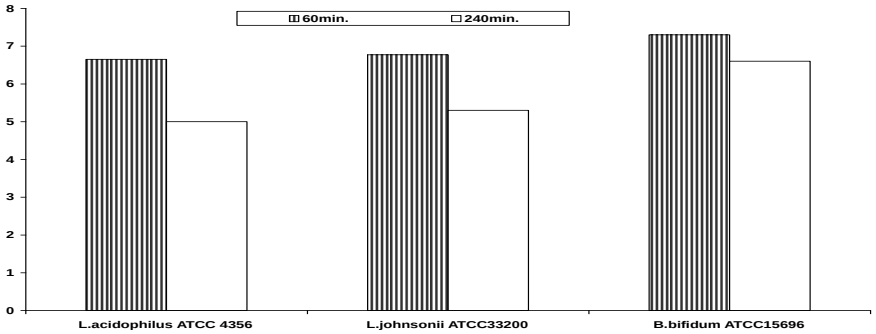


Fig. (3): Level of administered and recovered of three tested cultures tochicks.

Table (4) Growth of the three tested strains in coculture with E. coli : H7 ATCC 51657 in skim milk medium.

Tested strains		Incubation time (h)				
		Zero	12	24	36	48
E. coli	Log cfu/ml	4.48	5.30	6.64	6.78	7.30
	pH	6.7	6.2	5.9	5.7	5.3
L.acidophilus ATCC 4356 + E.coli	Log cfu/ml	4.60	4.00	3.47	2.84	1.90
	pH	6.7	5.9	5.4	5.0	4.8
L.johnsonii ATCC33200 + E.coli	Log cfu/ml	4.60	4.47	4.00	3.77	2.47
	pH	6.7	5.7	5.5	5.1	4.9
B.bifidum ATCC 15696 + E.coli	Log cfu/ml	4.60	3.90	3.00	2.30	1.69
	pH	6.7	5.8	5.7	5.5	5.1

CFu/g = Colony forming inhibition percent

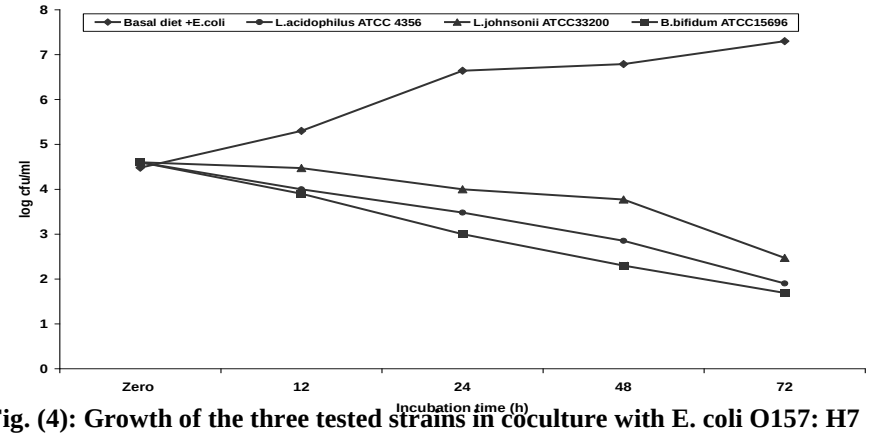


Fig. (4): Growth of the three tested strains in coculture with E. coli O157: H7 ATCC 51657 in skim milk medium.

Table (5) Growth inhibition % and survival % of the three tested strains in coculture with E. coli : H7 ATCC 51657 in skim milk medium.

Tested strains		Incubation time (h)			
		12	24	36	48
L.acidophilus ATCC 4356 + E.coli	G. inh (%)	13.04	24.35	38.04	58.70
	Survival %	86.96	75.65	61.96	41.30
L.johnsonii ATCC33200 + E.coli	G. inh (%)	2.83	13.04	18.04	46.30
	Survival %	97.17	86.96	81.96	53.70
B.bifidum ATCC 15696 + E.coli	G. inh (%)	15.22	34.78	50.00	63.26
	Survival %	84.78	65.22	50.00	36.74

G. inh (%) = Growth inhibition percent

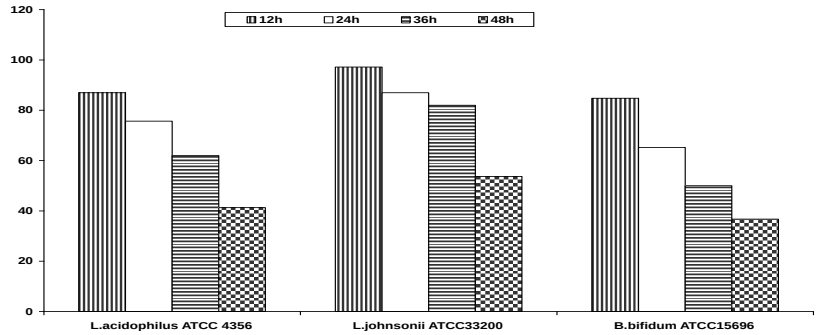


Fig. (5): Survival % of the three tested strains in coculture with *E. coli* O157: H7 ATCC 51657 in skim milk medium.

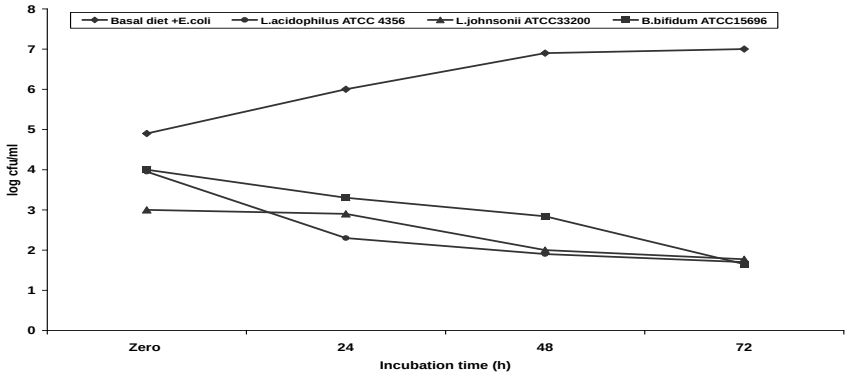
Table (6) Effect of feeding the three tested strains on the count of E. coli : H7 ATCC 51657 in the feces of Fyomy chicks.

Tested strains	Incubation time (h)						
	Zero	24		48		72	
	Log cfu/ml	Log cfu/ml	G. inh(%)	Log cfu/ml	G. inh (%)	Log cfu/ml	G. inh (%)
Basal diet + E.coli	4.90	6.00	-	6.90	-	7.00	-
Basal diet + L.acidophilus ATCC 4356 + E.coli	3.95	2.30	41.77	1.90	51.90	1.70	57.22
Basal diet+ L.johnsonii ATCC33200+E.coli	3.00	2.90	3.33	2.00	33.33	1.77	41.00
Basal diet + B.bifidum ATCC15696 + E.coli	4.00	3.30	17.50	2.84	29.00	1.65	58.75

CFu/g = Colony forming unit/ml.

G. inh (%) = Growth inhibition percent.

Fig. (6): Viable count of *E. coli* O157: H7 ATCC 51657 in the feces of Fayomy chicks administrated on the three tested strains.



References

AbouDawood, S. A. I.(2002).Survival of nonencapsulated and encapsulated*Bifidobacteriumbifidum* in probiotic Kareish cheese.Egyptian. J. Dairy Sci., 30(1):43-52.

Ahmed, K. A. (2009). Chemical and Biotechnological studies on probiotic starters. Ph.D. Thesis, Faculty of Agric., Al-Azhar Univ., Cairo, Egypt.

American Public Health Association (APHA) (1978). Standard methods for the examination of dairy products. 13th ed., APHA, New York, USA.

Badawi, R. M. and El –Sonbaty, A. H. (1997). Viability of *Staphylococcus aureus*and *Escherichia coli* in zabady made with bifidobacteria. Egypt. J. Dairy Sci., 25: 217 – 233.

Charteris, W.P.; Kelly, P.M.; Morelli, L. and Collins, J.K. (1998). Developmentand application of an in vitro methology to determine the transit tolerance of potentially probiotic *Lactobacillus* and *Bifidobacterium species* in the upper human gastrointestinal tract. Journal of Applied Microbiology, 84: 759-768.

Collins, J. K.; Thornton, G. and Sullivan, G. O. (1998). Selection of probiotic strains from human applications. Int. Dairy J., 8:487.

Dave, R.I. and Shah, N.P. (1996).Evaluation of media for selective enumeration of *Streptococcus thermophilus, Lactobacillus*

delbrueckii ssp. *bulgaricus*, *Lactobacillus acidophilus* and *bifidobacteria*.J. Dairy Sci., 79: 1529 – 1536.

David, B. and Michael, W. (2008). Probiotic in gastrointestinal disorders. Nelson Textbook of pediatrics. SaunersEsevier, Phladelephia, 18th ed., p:1618-1620.

Elhadidi, A.A. (2014). In Vitro And In Vivo Studies On Survival Of Some Probiotic Bacteria In Gastrointestinal Tract. J. of Home Economics, V. 24, No. (1).

Gibson, G.R. and Wang, X. (1994). Regulatory effects of bifidobacteria on the growth of other colonic bacteria. J. Appl. Bacteriol., 77 (4) : 412 – 420.

Grajek, W.; Olejnik, A. and Sip, A. (2005). Probiotics, Prebiotics and antioxidants as functional foods. ActaBiochim.Pol. 52: 665-671.

Guerra, N.P.; Bernardez, P.F.; Mendez, J.; Cachaldora, P. and Castro, L.P. (2007). Production of four potentially probiotic lactic acid bacteria and their evaluation as feed additives for weaned piglets. J. Animal Feed Science and technology, 134: 89 – 107.

Havenaar, R.; Brink, and Huis, J.H.J (1992). Strains forprobiotic use.Probiotics, the Scientific Basis, fuller, pp. 209-224, London, uk. :Chapman and Hall.

Helland, MH.;Wicklund, T. and Narvhus, IA. (2004). Growth and metabolism of selected strains of probiotic bacteria in maize porridge with added malted barley.Int. J. Food Microbiol. 91: 305-3013.

Hosono, A.; Yatsuki, K. and Tokita, F. (1977).Isolation and characterization of on inhibitory substances against Escherichia coli produced by Lactobacillus cidophilus.Milchwissenschaft32 : 727 – 730.

Kebary, K.M.K. (1995). Production, Partial Purification and stability of antimicrobial substances produced by *Bifidobacteriumbifidum DI*. Egypt. J. Dairy Sci., 23 : 2, 151 – 166.

Kebary, K.M.K.; Moussa, A.M.; Hamed, A.I. and Yousef, E.T. (1996).Quality of zabady made with *Bifidobacteriumbifidum DI.* The Proceeding of 36th Science week. Syria.

Kershah, T.A. A. (2014).Studies on probiotic bacteria. M.Sc. Thesis, Faculty of Agric., Al-Azhar Univ., Cairo, Egypt.

Makras, L. and De Vuyst, L. (2006).The in vitro inhibition of gram-negative pathogenic bacteria by *bifidobacteria* is caused by the production of organic acids. International Dairy Journal, 16:1049-1057.

Man, J.C. ;deRogosa, M. and Sharp, M.F. (1960). A medium for the cultivation of *lactobacilli*. Journal of Applied Bacteriology, 23: 130-135.

Mathara J. M.; Schillinger U.; Guigas C.; Franz C.; Kutima P.M.; Mbugua S. K.; Shin H.-K. andHolzapfel, W. H. (2008). Functional characteristics of *Lactobacillus spp.*From traditional Maasai fermented milk products in Kenya. International Journal of Food Microbiology, 60: 253.

Misra, A. K. and R. K. Kuila (1994b).Effect of bifidus milk feed on excretory pattern of coliforms. Indian I. Dairy Sci., 47:531.

Oh, S.; Kim, S.H. and Wor, R.w. (2000). Characterization and purification of a Bacteriocin produced by a potential probiotic culture, *Lactobacillus acidophilus* 30SC. J. Dairy Sci., 83: 2747-2752.

Parvez, S.; Malik, KA; Ah Kang, S. and Kim, HY. (2006). Probiotics and their fermented food products are beneficial for health. J. Appl. Microbiol. 100: 1171-1185.

Patricia, RM.; Jeroen, H. and Pieternela, Z. (2002).An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. Int. Dairy J. 12: 163-171.

Shah, N. P. (2000). Probiotic bacteria: selective enumeration and survival in dairy foods. J. Dairy Sci. 83: 894-907.

Sreekumar, O. and Hosono, A. (2000).Immediate effect of *Lactobacillus acidophilus* on the intestinal flora and fecal enzymes of rats and the in vitro inhibition of *Esherichia coli* in coculture. J. Dairy Sci. 83: 931-939.

Sultana, K., Godward, G., Reynolds, N., Arumugaswamy, R., Peiris, P. and Kailasapathy, K. (2000).Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt. International Journal of Food Microbiology 62: 47-55.

Terzaghi, B. E. and W. E. Sandine, (1975). Improve medium for lactic streptococci and their bacteriophages. Appl. Microbiol., 29:807.

Vijayendra, S.V. N. and Gupta, R. C. (1992).Therapeutic importance of *bifidobacteria* and *Lactobacillus acidophilus* fermented milks.Indian Dairy Man, 44; 595.

Wijsman, M.R.; Hereijgers, J. M. and De-Groote, F. H. (1989).Selective enumeration of *bifidobacteria* in fermented dairy products. Neth. Milk Dairy J., 43: 395.

Journal of Home Economics, Volume 24, Number (1), 2014

إختيار بعض سلالات bifidobacteria, lactobacilli كمزارع مدعمات حياة محتملة

خاطر عبد الفتاح أحمد خاطر
قسم الألبان كلية الزراعة - جامعة الأزهر بالقاهرة

لدراسة مدى امكانية استخدام سلالات Lb. johnsonii ATCC 33200, Lb. acidophilus ATCC 4356, B. bifidum ATCC 15696 كمدعمات حياة تم اختبار هذه السلالات معمليا وتطبيقيا لمدى قدرتها على تحمل كل من العصير المعدى والمعوى وتحمل ظروف القناة الهضمية الصعبة والمرور خلالها بالاضافة إلى قدرتها على التأثير المضاد لبكتيريا E.coli O157:H7.

تم اختبار السلالات الثلاثة معمليا لمعرفة مدى قدرتها على تحمل كل من العصارة المعدية والمعوية حيث أوضحت النتائج قدرة تلك السلالات على العالية المقاومة والتى تراوحت ما بين 93.95 ٪ إلى 98.75 ٪ فى العصارة المعدية بينما كانت تلك النسبة ما بين 84.07 ٪ إلى 94.48 ٪ فى العصارة المعوية.

كما أجريت تجربة تطبيقية بتغذية 3 مجموعات من الدواجن الفيومى على العليقة الجافة مضاف إليها أحد هذه السلالات المختبرة، وبعد ساعتين من التغذية تم ذبح الطيور وإجراء عد لهذه البكتيريا فى الأمعاء الدقيقة. أكدت النتائج المتحصل عليها قدرة السلالات الثلاثة المختبرة على تحمل الظروف الصعبة للقناة الهضمية والمرور خلالها وكانت بكتيريا B. bifidum ATCC 15696 الأكثر مقاومة بنسبة 90.41 ٪.

كما تم إختبار السلالات الثلاثة لمدى قدرتها على التأثير المضاد لبكتيريا E. coliمعمليا وذلك بالتنمية المشتركة بين بكتيريا E. coli وكل من تلك السلالات المختبرة منفردة وذلك فى بيئة اللبن ولمدة 48 ساعة. وأوضحت نتائج التجربة التأثير المضاد القوى لبكتيريا B. bifidum ATCC 15696 على بكتيريا E. coli حيث إنخفضت أعداد E. coli بمقدار 2.91 دورة لوغاريتمية وتلاها فى التأثير سلالة Lb. acidophilus ATCC 4356 حيث إنخفضت أعداد E. coli بمقدار 2.7 دورة لوغاريتمية.

فى التجربة التطبيقية لدراسة تأثير كل من السلالات المختبرة على بكتيريا E. coli تم إجراء تجربة تغذية لمدة 3 أيام بأستخدام 4 مجموعات من الدجاج الفيومى مع إجراء عد يومى لمحتويات إخراج الطيور من بكتيريا E. coli. أظهرت النتائج زيادة أعداد بكتيريا E. coli فى عينات مجموعة الكنترول (التغذية على عليقة جافة + E. coli بتركيز 10^3 خلية / جرام) حيث ارتفعت الأعداد من 10^4 خلية / جرام إلى 10^7 خلية / جرام فى نهاية التجربة ، فى حين تناقصت أعداد بكتيريا E. coli فى المجاميع الثلاثة الأخرى (التغذية على عليقة جافة + E. coli بتركيز 10^3 خلية / جرام + السلالة المختبرة بتركيز 10^7 خلية / جرام) مما يعكس تأثير مضاد لتلك السلالات على بكتيريا E. coli ، وكانت سلالة B. bifidum ATCC 15696 الأكثر تأثيرًا حيث تناقصت أعداد الـ E. coli بنسبة 58.75 ٪ يليها سلالة Lb. acidophilus ATCC 4356 بنسبة تناقص 57.22 ٪ ويعزى ذلك إلى قدرة تلك السلالات على إنتاج المواد المضادة لنمو بكتيريا E. coli مثل حمض الخليك واللاكتيك والبيفيدين والأسيدوفلين.

من النتائج السابقة يمكن ترشيح تلك السلالات لأستخدامها كمدعمات حياة نظرًا لقدرتها على تحمل الظروف الصعبة للقناة الهضمية من pH منخفض وأنزيمات بالإضافة إى قدرتها على القضاء على البكتيريا المرضية مثل E. coli وما تسببه من إضطرابات هضمية.