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ASSESSMENT OF PROBIOTIC PROPERTIES OF SOME MICROBIAL IN SIMULATED GASTROINTESTINAL TRACT ENIVRONMENT

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ABSTRACT

The viability of free and microencapsulation *L. acidophilus* La-5, *L. casei* - 01, *L. helveticus* LhB 02, *B. bifidum* Bb-12, *K. lactis* NRRL Y- 8279 and *Sacch. cervisiae* DSMZ 70449 cells in simulated gastrointestinal tract environment was determined. The viable cells of strains decreased significantly except *B. bifidum* and *K. lactis* in microencapsulated form as compared with their initial count in the presence of lysozyme (100 µg/ ml). The viability of all strains at pH 1.5 was less than that at pH 3.0 throughout exposure time (180 min). By increasing the concentration of bile salt, the resistance of all microorganisms decreased and there was a significant difference between their initial count and that after 180 min at 37ºC. Bile salt hydrolase (BSH) activity was detected with *L. acidophilus* and *B. bifidum.* The viability of all strains decreased with the increase of phenol concentration and exposure time. The release of viable cells from microcapsules in simulated colonic pH solution increased significantly as the exposure time increased from zero to 90 min at 37ºC. Also strains were resistant to all antibiotics used in this study except yeast strains were susceptible to neomycin. Results of this study showed considerable difference in the percentage of hydrophobicity between strains. Generally the final number of viable cells of the strains was still above the levels suggested to produce their claimed health benefits. On the other hand the microencapsulation of strains protected them against adverse effects of GIT environment and enhanced their survivability.

INTRODUCTION

Probiotic bacteria are live microbial strains that, when applied in adequate doses (10^6 – 10^7), beneficially affect the host animal by improving its intestinal microbial balance (Fooks *et al*.1999; and Ouwehand *et al*., 1999). The probiotic strains used in dairy products most commonly belong to *Lactobacillus* and *Bifidobacterium* genera. In addition, some species of streptococci, enterococci, and yeasts are considered probiotics (Ishibashi and Yamazaki 2001; and Gotcheva *et al*.,2002). The consumption of probiotic cultures positively affects the composition of gastrointestinal tract (GIT) microflora and extends a range of host benefits which so far claimed to be: pathogens interference, immune system stimulation and immunomodulation, anticarcinogenic and antimutagenic activities, alleviation of symptoms of lactose intolerance, reduction in serum cholesterol, reduction in blood pressure, decrease incidence and duration of diarrhea, prevention of vaginitis and maintenance of mucosal integrity (Stanton *et al*.,1998; Fooks *et al*.,1999;and Ouwehand *et al*.,1999 and Liong and Shah, 2005).

Probiotic bacteria selected for commercial use in foods and in therapeutics must retain the characteristics for which they are originally selected. These include characteristics for growth and survival during

manufacture and after consumption, and during transit through the gastrointestinal tract (GIT). The initial screening and selection of probiotics includes testing of the following important criteria: safe history of origin and/or use; ability to grow quickly and survive incorporation into a wide variety of food matrices; maintaining their viability during the storage period of products; acid and bile tolerance; bile metabolism; intestinal epithelial adhesion properties; production of antimicrobial substances; antibiotic resistance patterns; ability to inhibit known gut pathogens, spoilage organisms, or both; and immunogencity. Its important also to assess the use of the probiotic strain for other uses e.g., as a prevention agent, a detoxification agent and so on. These properties can be determined by in vitro assays that are generally used for the selection of potential probiotics (Bernardeau *et al.,* 2001; Tuomola *et al.,* 2001; and Salminen *et al.,* 2004).

Microencapsulation of probiotics offer a potential way of improving the survival of probiotics during manufacture and storage of functional dairy products and during GIT transition (Shah and Ravula, 2000; Adhikari *et al*., 2003; Agustin, 2003 and Picot and Lacroix, 2004). Microencapsulation using gelatin or vegetable gum provides protection to acid sensitive Bifidobacteria; however, the most widely used matrix for microencapsulation is alginate. Alginate beads have been found to increase the survival of probiotics by up to 80-95% (Krasaekoopt *et al.,* 2003). Encapsulation of *L.rhamnosus* in alginate improved survival at pH 2.0 up to 48h, while the free cells were destroyed completely (Goderska *et al*., 2003).

Therefore, the objectives of this study were to assess some probiotic properties of some lactic acid bacteria, *Bifidobacterium* and yeast strains and to study the effect of microencapsulation on their viability.

MATERIALS AND METHODS

L. acidophilus La-5, *L. casei* - 01, *L. helveticus* Lh. B 02, and *B. bifidum* Bb-12 were obtained from Chr. Hansens' Laboratories, Copenhagen, Denmark. *K. lactis* NRRL Y- 8279 was obtained from Northern Regional Research Laboratory, USA. *Sacch. cerevisiae* DSMZ 70 449 was obtained from Deutsche Sammlung von Mikroorganism und Zellkulturen GmbH, German.

All microbiological media (MRS and Malt extract agar) used were obtained from Oxoid Division of Oxiod LTD, London.

Pepsin, egg white lysozyme, L- cystein hydrochloride, lithium chloride, Sodium tauroglycocholate, sodium salt taurodeoxycholic acid, and phenol were obtained from Sigma Chemical Co., USA. Anaerogen shachets were obtained from Oxoid Ltd., Basingstoke, Hampshire, England. Ampecilline, chloramphincol, erythromycin, tetracycline and neomycin were obtained from Egyptian pharmaceutical chemicals Co. of Nile, El Naser, Kahira, Chemical industries development and Memphis respectively. Vancomycin and n-hexadecane were obtained from Merck Co., Germany. Sodium alginate was obtained from MIFAD Co., for food industries, Egypt.

- Millipore filter 0.45 µm were obtained from Whatman Co. USA.

Direct Vat Set (DVS) lactobacilli and bifidobacteria strains were transferred at rate of 2 % into MRS broth supplemented with L-cystein hydrochloride 0.05% (MRS-C) and incubated aerobically for lactobacilli or anaerobically for bifidobacteria at 37ºC for 18 h. However, lyophilized yeast strains were subcultured two times consequently prior to use in sterile malt extract (ME) broth and incubated aerobically at 37 ◌ْC for 18 h.

As with the free cell pellets preparation the activated cultures were centrifuged at 3000 ×g for 5 min at 4˚C then pellets were harvested, washed twice and suspending in normal saline (Mandal *et al*., 2006).

Microorganisms were microencapsulated in 3% sodium alginate matrix as described by Sheu and Marshall (1993). Cells were microentrapped by mixing one part culture concentrate with four parts sodium alginate (3%). One part of the mixture was then added dropwise to 5 parts vegetable oil (250 ml in an 800 ml beaker) containing Tween 80 (0.2%), which was stirred at 200 rpm by magnetic stirring. Within 10 min, an uniformly turbid emulsion was obtained with no evidence of a free aqueous phase. Calcium chloride (~500 ml 0.05M) was added quickly but gently (20 ml/sec) down the side of the beaker until the water/oil emulsion was broken. Calcium alginate beads were formed within 10 min. The beads were collected by gentle centrifugation (350 ×g for 10min at 4˚C) and washed with sterile water.

Simulated gastric juice was prepared by suspending pepsin (3g/l) in saline (0.5%, v/v) and adjusting the pH to 1.5 or 3 with 12N HCl. It was then sterile- filtered through a millipore filter 0.45 µm (Lian *et al*., 2003)

Assessment of probiotic criteria.

1. Lysozyme resistance:

The free or microencapsulated cells (1g) was suspended in 9 ml of 0.06 mol/l Phosphate buffer saline (PBS) pH 6.2 supplemented with1 % NaCl then 100 µg/ml lysozyme was added. PBS cell suspensions (0.06 mol/l PBS pH 6.2) without lysozyme were served as a control (Kimoto *et al.*, 2000). The cell suspensions were incubated at 37◌ْ C for 3 h and the viable cells were enumerated at 0, 90 and 180 min.

2. Simulated gastric juice resistance:

The free or microencapsulated cells (1g) was suspended in 9 ml of simulated gastric juice pH 3 or 1.5 and incubated at 37◌ْC for 3h. The viable cells were enumerated at 0, 90 and 180 min (Lian *et al.,* 2003).

3. Bile salt resistance:

The free or microencapsulated cells (1g) was suspended in 9 ml of sodium tauroglycocholate solution at concentration of 0.1, 0.3, 0.5, and 2 % then incubated at 37◌ْC for 3h. The viable cells were enumerated at 0, 90 and 180 min (Ibrahim and Bezkorovaing, 1993).

4. Bile salt hydrolase (BSH) activity:

The strains were screened for BSH activity by spotting 10 µl aliquots of overnight cultures (10^7 cfu/ml) on MRS plates for lactobacilli, MRS. L. cysteine-Lithium chloride for *Bifidobacterium* and malt extract agar for yeast strains. All media supplemented with 0.5% (w/v) sodium salt of taurodeoxycholic acid and 0.37 g/L $CaCl_2$ (Dashkevicz and Feighner, 1989).

Plates were incubated anaerobically for 72 h at 37°C and strains forming precipitation zones were regarded BSH positive.

5. Phenol tolerance:

The free or microencapsulated cells (1g) was suspended in 9 ml phenol solution at concentration of 0.1, 0.3 and 0.5 then incubated at 37° C for 3 h. The viable cells were enumerated at 0, 90 and 180 min.

6. Antibiotic resistance:

Antibiotic sensitivity of the investigated microbial strains was tested using the disc assay method according to Charteris *et al*. (1998b). The used antibiotics were Ampecilline, Chlorphincol, Erythromycin, Tetracycline, Neomycin, and Vancomycin.

7. Release of microencapsulated cells in simulated colonic solution:

Microcapsules (1g) of different strains were transferred into 10 ml simulated colonic pH solution (0.1 M KH_2PO_4, pH 7.4 ± 0.2), mixed gently and incubated at 37° C for 90 and 180 min, aliquots (1ml) were taken and viable counts were enumerated (Mandal *et al.*, 2006).

8. Cell surface hydrophobicity:

The test for microbial adhesion to hydrocarbons was adopted to screen lactobacilli, bifidobacteria and two yeast strains for cell surface hydrophobicity (Doyle and Rosenberg, 1995). Cells from 5 ml of each culture were collected by centrifugation at 9500 g at 4° C for 6 min. Cells were washed twice with quarter-strength Ringer's solution (QSRS).One milliter of cell suspension (in QSRS) was used to determine the OD_{580} (reading 1). In duplicate assessments, a further1.5 ml of this suspension was added to an equal volume of n. hexadecane, organic phase and thoroughly mixed for 2 min using a vortex. The phases were allowed to separate at room temperature for 30 min, after which 1 ml of the watery phase was removed and the OD_{580} was determined (reading 2). The OD_{580} of duplicate assessments was averaged and used to calculate hydrophobicity:

% Hydrophobicity = [(OD_{580} reading 1- OD_{580} reading 2) / OD_{580} reading 1] x 100.

Methods of analysis:

Single strains of Lactobacilli and yeast strains were enumerated on MRS agar and malt extract agar respectively (Oxoid Manual, 1982). *B. bifidum* was enumerated on modified MRS agar supplemented with 0.05% L- cysteine hydrochloride and 0.3 % lithium chloride (Dave and Shah, 1996). The plates of single lactobacilli and bifidobacteria strains incubated aerobically and anaerobically respectively at 37 ºC for 72 h. The plates of yeast strains were aerobically incubated at 25 ºC for 5 days.

The entrapped cells were released from the gel according to Sheu and Marshall (1993). Microencapsulated cells (1g) were suspended in 10 ml phosphate buffer (0.1M, pH 7.4) followed by gentle shaking for 30 min. The number of released cells was determined by plate count using media as previous.

The values of all experiments are presented as the means of triplicate analysis. Statistical analysis for obtained data was carried out using the Statistical Analysis System (SAS, 1994).

RESULTS AND DISCUSSION

1. Lysozyme resistance:

There are certain enzymes in gastrointestinal tract which are harmful to microorganisms. One of these is lysozyme which can lyse gram positive bacterial cells. Table (1) shows the viability of some free and microencapsulated microorganisms in the presence of lysozyme at concentration of 100 µg/ ml. The lysozyme concentration used in this assay is higher than the physiological intestinal lysozyme concentration (Suskovic *et al*., 1997).At this concentration of lysozyme and after 180 min., the logarithm of the viable cells count of free and microencapsulated microorganisms decreased significantly except *B. bifidum* and *K. lactis* in microencapsulated form as compared with their initial count. However, the viable cells count of all microorganisms in both forms remained higher than 10^6 cfu/ml after 180 min at 37 ◌̊ C. Data obtained also show the positive effect of microencapsulation as it enhanced the microorganisms survivability. Gilland (1979) showed that mainly gram positive bacteria are sensitive to lysozyme, but that genera *Lactobacillus and streptococcus* are more resistant than other gram positive bacteria. Also, Abd El-Salam *et al*. (2004) showed that among nine strains of lactobacilli exposed to lysozyme at the same concentration, most *Lactobacillus* strains slowly decreased until time exposure 90 min.

Table (1):Viability of some microorganisms in the presence of lysozyme.

Strains	Initial Count		Exposure time (min) at 37˚C					
			0		90		180	
	Free	Caps	Free	Caps	Free	Caps	Free	Caps
	Log cfu/ml							
L. acidophilus La-5	8.30	8.28	8.25	8.26	8.15	8.20	7.75	8.00
L. casei 01	8.45	8.40	8.40	8.36	8.00	8.20	7.90	8.08
L. *helveticus* Lh. B 02	8.40	8.30	8.30	8.26	7.48	7.70	6.18	6.48
B. *bifidum* Bb-12	8.30	8.26	8.26	8.20	8.18	8.15	8.00	8.10
K. lactis NRRL Y- 8279	8.18	8.11	8.15	8.08	7.90	8.00	7.65	7.95
Sacch. cerevisiae DSMZ 70 449	8.11	8.08	8.00	7.95	6.85	7.08	6.48	6.70

$LSD_{0.05}$ of experimental treatments = 0.22 (*P* <0.05)

2. Simulated gastric juice resistance:

Acidic conditions in the stomach are a natural barrier preventing most microorganisms from passing into the intestine. Normally, pH in the stomach of healthy individuals is lower than 3.0 (Gotcheva *et al*., 2001) but the pH reaches to 1.5 when fasting (Tumola *et al*., 2001) .Many strains of lactobacilli and bifidobacteria intrinsically lack the ability to survive harsh conditions in the gut and may not be suitable use as dietary adjuncts in fermented foods (Lankaputhra and Shah, 1995).

In order to evaluate the suitability of some free and microencapsulated microorganisms to stomach conditions, 6 strains were exposed to simulated gastric juice at pH 1.5 and 3.0 and the viability of all strains was assed after

90 and 180 min. at 37 ºC. As shown in Table (2), the viability of all strains (free and microencapsulated) at pH 1.5 was less than those at pH 3.0 throughout the exposure time being at a higher rate with the free form. *L. casei* and *B. bifidum* showed high survivability compared with *L. acidophilus* and *L. helveticus* in both forms at pH 1.5 or 3.0 during 3 h exposure time. *L. casei* (free or microencapsulated) showed non significant decrease in numbers at pH 3.0 and 1.5 at the end of exposure time. Also, data show the effectiveness of microencapsulation as a protective mean against the depressive effect of low pH on the survivability of microorganisms as the reduction rate in the population of *L. helveticus, L. acidophilus* and *Sacch. cerevisiae* is lower in microencapsulated form than in the free one especially after exposure time 180 min at pH 1.5 and 3.0. These results were confirmed by Playne (1994) who reported that *L. acidophilus* did not grow well below pH 4.0. Lankaputhra and Shah (1995), however, reported that the studied six strains of *L. acidophilus* survived well at pH 3.0 or above and the viable count remained above 10^7 cfu/ml after 3 h incubation. Also literature pointed out *L. casei* cultures were more adaptable to the acidic environment (Vinderola *et al*., 2000). As for yeast strains, the viability of *Sacch. cerevisiae* showed a significant decrease. However, *K. lactis* showed more resistance than *Sacch. cerevisiae* in both forms at pH 3 and 1.5 as shown in the same Table.

Table (2): Viability of some microorganisms in presence of simulated gastric juice.

Strains	Cell forms	Initial Count	Exposure time (min) at 37˚C					
			zero		90		180	
			pH					
			3.0	1.5	3.0	1.5	3.0	1.5
		Log cfu/ml						
L.acidophilus LA-5	Free	8.30	8.26	8.20	7.20	6.18	5.78	5.30
	Caps	8.28	8.23	8.22	8.08	7.00	7.30	6.70
L. casei 01	Free	8.45	8.40	8.30	8.20	8.08	8.00	7.90
	Caps	8.40	8.34	8.20	8.28	8.00	8.15	7.95
L. helveticus Lh.B 02	Free	8.40	8.18	7.90	7.00	6.00	6.16	5.30
	Caps	8.30	8.00	7.80	7.90	7.65	7.80	7.50
B. bifidum Bb-12	Free	8.30	8.28	8.18	8.23	8.11	8.18	7.50
	Caps	8.26	8.23	8.18	8.20	8.11	8.18	7.50
K. lactis NRRL Y 8279	Free	8.18	8.11	7.90	8.00	6.70	7.70	7.30
	Caps	8.10	8.00	7.70	7.90	7.30	7.80	7.20
Sacch.cerevisiae DSMZ 70449	Free	7.90	7.90	7.70	6.70	6.70	6.65	6.00
	Caps	8.08	8.00	7.85	7.90	7.78	7.30	7.08

$LSD_{0.05}$ of experimental treatments = 0.66 (*P* <0.05)

3. Bile salt resistance:

After passage through the acidic stomach conditions, it is important that, for application of LAB strains as probiotics, they are able to survive the bile salt in the intestine, the normal level of which is around 0.3%, but may be range up to the extreme 2.0% during the first hour of digestion, most researchers assess bile resistance within the range 0.1- 0.5% (Gotcheva *et*

al., 2002). The probiotic bacteria must reach the colon in large quantities to facilitate colonization and to exert a beneficial effect on host. Therefore the strain is considered sensitive to bile salt if the population decreased to $<10^5$ cfu/ ml (Kim, 1988)

Table (3) illustrates the viability of some microorganisms in the presence of different concentrations of bile salt at different exposure time. At 0.

Table (3): Viability of some microorganisms in bile salt solution with different concentrations.

Strains	Initial count Log cfu/ml		Bile Salt conc.%	Exposure time (min) at 37˚C					
				0		90		180	
	Free	Caps		Free	Caps	Free	Caps	Free	Caps
				Log cfu/ml					
L. acidophilus La-5	8.45	8.40	0.1	8.40	8.35	8.20	8.28	8.00	8.15
			0.3	8.35	8.30	7.30	7.90	6.30	7.70
			0.5	8.30	8.28	6.00	7.00	5.30	6.00
			2	8.30	8.28	5.30	6.00	<5	5.20
L. casei 01	8.30	8.28	0.1	8.28	8.28	8.00	8.15	7.95	8.00
			0.3	8.25	8.20	7.90	8.00	7.30	7.70
			0.5	8.00	8.15	6.30	6.90	5.30	6.30
			2	8.00	8.00	5.70	6.00	5.00	5.60
L. helveticus Lh. B 02	8.40	8.30	0.1	8.40	8.30	7.90	8.15	6.30	7.00
			0.3	8.30	8.25	6.30	6.70	5.30	6.30
			0.5	8.00	8.15	5.00	6.30	<5	5.60
			2	8.00	8.15	<5	5.30	<5	5.00
B. bifidum Bb- 12	8.30	8.26	0.1	8.30	8.20	8.00	8.15	7.90	8.00
			0.3	8.20	8.20	7.90	8.00	7.30	7.70
			0.5	8.00	8.00	6.60	7.30	6.00	6.70
			2	8.15	8.00	5.90	6.00	5.30	6.00
K. lactis NRRL Y 8279	8.18	8.15	0.1	8.15	8.00	7.90	8.00	7.65	7.90
			0.3	8.15	8.08	7.65	7.90	7.00	7.65
			0.5	8.00	8.00	7.30	7.00	6.70	7.30
			2	8.15	8.00	7.00	7.30	6.30	7.00
Sacch.cerevisie DSMZ 70449	8.18	8.15	0.1	8.00	8.00	7.00	7.30	6.60	6.90
			0.3	7.90	8.00	6.30	7.00	6.00	6.60
			0.5	7.90	7.90	6.00	6.60	5.30	6.30
			2	7.95	7.90	5.30	6.30	5.00	6.00

$LSD_{0.05}$ of experimental treatments = 0.57 (*P* <0.05)

1% bile salt concentration there was no significant effect on the viability of all free and microencapsulated strains as the population was $> 10^7$ cfu/ml after 180 min at 37ºC except *L. helveticus* Lh. B 02 and *Sacch. cerevisiae* DSMZ 70449. By increasing the concentration of bile salt to 0.3%, the resistance of all free and microencapsulated microorganisms decreased and there was a significant difference between their initial count and those after 180 min at 37ºC. However, the viable cells count of all microorganisms remained higher than 10^6 cfu/ml. At 0.5% bile salt concentration the population of free *L. helveticus* Lh. B 02 decreased to less than 10^5 cfu/ml after increasing the exposure time to 180 min as shown in the same Table. At 2% bile salt concentration the free and microencapsulated *B. bifidum* Bb-12 and *L. casei*

01 showed the highest resistant between the bacterial strains as they tolerate this concentration of bile salt for 180 min. On the other hand the population of free *L. helveticus* and *L. acidophilus* decreased to less than 10^5 cfu/ml after increasing the exposure time to 180 min. As for *K. lactis* NRRL Y 8279 and *Sacch. cerevisiae* DSMZ 70449, the results in Table (3) indicate that the *Sacch. cerevisiae* was more sensitive to bile salt than *K. lactis*. The foregoing results indicate that the influence of microencapsulation was effective for all the studied strains especially with the higher concentration of bile salt and by expanding the exposure period to 180 min.

These results are in agreement with those reported by Ibrahim and Bezkorovaing (1993) who reported that *Bifidobacterium* species are able to survive well in presence of 0.3 % sodium tauroglycocholate. Lankaputhra and Shah (1995) showed that some microbial strains kept count unaffected after 3 h of incubation at these bile levels. Charteris *et al.* (1998a) showed good bile tolerance (0.3 % v/v) of 7 *Lactobacillus* and 7 *Bifidobacterium* strains in an in vitro study. Gotcheva *et al*. (2002) showed that *Candida rugosa* Y28 and *Candida lambica* Y30 were more resistant to bile salt than LAB. Lian *et al.* (2003) showed that microencapsulation of *Bifidobacterium* strains could enhance their survivability in presence of bile salt.

4. Bile salt hydrolase activity:

Bile salt hydrolytic (BSH) activity may contribute to resistance of LAB to the toxicity of conjugated bile salts in the duodenum and therefore is an important colonization factor and prerequisite for strains to be effective probiotic microorganisms. BSH activity can be determined by *in vitro* assays that are generally used for the selection of potential probiotic bacteria (Schillinger *et al*., 2005).

Data presented in Table (4) show the BSH activity of *Lactobacillus*, *Bifidobacterium* and two yeast strains. It is obvious from the obtained results that *L. acidophilus* La-5 and *B. bifidum* Bb-12 produced precipitation zones in the BSH plates assay whereas *L. casei* -01, *L. helveticus* LhB 02, *K. lactis* NRRL Y- 8279 and *Sacch. cerevisiae* DSMZ 70 449 were BSH negative. Similar results for lactobacilli were previously reported by Deshkevicz and Feihner (1989) who didn't detect BSH activity of seven strains of *L. casei* (most probably, *L*. *paracasei*). Also Moser and Savage (2001) didn't observe *L. rhamnosus* and *L. paracasei* strains able to produce BSH. However, both studies reported that most strains of the *L. acidophilus* group isolated (human / animal and dairy isolates) were BSH positive.

Table (4): Bile salt hydrolase activity of some microorganisms.

Strains	BSH activity
L. acidophilus La-5	+
L. casei - 01	-
L. *helveticus* Lh. B 02	-
B. *bifidum* Bb-12	+
K. lactis NRRL Y- 8279	-
Sacch. cerevisiae DSMZ 70 449	-

5. Phenol tolerance:

Phenol is an intermediate of putrefactive processes in the GIT and may be formed by the bacterial deamination of some aromatic amino acids derived from dietary or endogenously produced proteins (Suskovic *et al*., 1997). So high tolerance towards phenol is very an important criterion in the selection of microbial strains for the probiotic use.

Therefore the effect of different concentrations of phenol during an exposure time of 180 min on the survival of some microorganisms was studied and the results are shown in Table (5). It is noticeable that by increasing the concentration of phenol from 0.1 to 0.5 % and the exposure time up to 180 min the survival of the strains tested in both forms free and microencapsulated decreased with no significant difference between the microencapsulated and free forms. Data also show that *L. helveticus* Lh.B 02 is the most sensitive strain to the presence of phenol as it can tolerate only a concentration of 0.1% for only 90 min of exposure, then the log cfu/ml decreased to <5 after 180 min of exposure followed by *L. acidophilus* La-5 which can tolerate the presence of the same concentration of phenol but for longer period (180 min).

Table (5): Viability of some microorganisms in phenol solution with different concentrations.

Strains	Initial count Log cfu/ml		Phenol conc.%	Exposure time (min) at 37˚C					
				0		90		180	
	Free	Caps		Free	Caps	Free	Caps	Free	Caps
						Log cfu/ml			
L. acidophilus La-5	8.45	8.40	0.1	8.40	8.35	6.65	6.90	6.00	6.30
			0.3	8.30	8.28	<5	<5	<5	<5
			0.5	8.28	8.20	<5	<5	<5	<5
L. casei 01	8.30	8.28	0.1	8.28	8.25	7.30	7.65	6.65	7.00
			0.3	8.25	8.20	6.30	6.90	5.30	5.60
			0.5	8.15	8.18	<5	<5	<5	<5
L. helveticus Lh. B 02	8.40	8.30	0.1	8.30	8.25	5.30	5.65	<5	<5
			0.3	8.15	8.20	<5	<5	<5	<5
			0.5	8.00	8.15	<5	<5	<5	<5
B. bifidum Bb-12	8.30	8.26	0.1	8.20	8.18	7.90	8.00	7.60	7.90
			0.3	8.15	8.00	6.30	6.70	5.00	5.15
			0.5	8.00	8.00	<5	<5	<5	<5
K. lactis NRRL Y 8279	8.18	8.15	0.1	8.00	7.90	7.00	7.30	6.90	7.00
			0.3	8.00	8.00	6.30	6.60	5.30	5.40
			0.5	7.90	8.00	5.15	5.30	<5	<5
Sacch.cerevisie DSMZ 70449	8.00	8.08	0.1	8.00	8.00	6.90	7.30	6.00	6.30
			0.3	8.00	8.08	6.00	6.30	5.00	5.20
			0.5	8.00	8.00	5.00	5.15	<5	<5

$LSD_{0.05}$ of experimental treatments = 0.60 (*P* <0.05)

On the other hand, *B. bifidum* Bb-12 was the most tolerant microorganisms as it has the highest degree of survival in the presence of 0.1% of phenol after exposure for 3h, followed by *K lactis* NRRL Y 8279 and *L. casei* 01 then *Sacch. cerevisiae* DSMZ 70449. Furthermore, by increasing the concentration of phenol to 0.3% there was no significant difference in the

survival between *B. bifidum*, *L. casei*, *K. lactis* and *Sacch cerevisiae* after exposure for 90 and 180 min. Regarding the highest concentration of phenol used in this study (0.5%), only *K. lactis* and *Sacch. cerevisiae* were capable for growing well after exposure period of 90 min. Therefore, the effect of different concentrations of phenol during an exposure period of 180 min. on the survival of some microorganisms has studied and the results are shown in Table (5). These results are partly in agreement with Suskovic *et al* (1997) who found that *L. acidophilus* M92 can grow well in the presence of phenol. Abd EL-Salam *et al.* (2004) also reported that *L. acidophilus* TISTR 450, *Lb. Johnsonii* ATCC 33200 and *Lb. acidophilus* ATCC 20552 strains exhibited a good phenol tolerance.

6. Antibiotic resistance:

The intestinal microbial balance can be disturbed by using of antibiotic in the therapy either gastrointestinal tract or urogenital tract. Antibiotic resistance of probiotic strains assures maintenance of healthy intestinal microbiota throughout antibiotic treatments of microbial infections.

In this study *L. acidophilus* La-5, *L. casei* - 01, *L. helveticus* Lh. B 02, *B. bifidum* Bb-12, *K. lactis* NRRL Y- 8279 and *Sacch. cerevisiae* DSMZ 70 449 were assayed for their susceptibility to six antibiotics, including inhibitors of cell wall synthesis (Ampecilline and Vancomycin), protein synthesis (Chloramphincol, Erythromycin and Tetracyclin), and cell wall synthesis for eukaryotes (Neomycin). Data presented in Table (6) show that all bacterial strains were resistant to all antibiotics which used in this study. However, the two yeast strains exhibited antibiotic susceptibility profiles to neomycin and resistant to other antibiotics. The same trend was found by Charteris *et al.* (1998) who demonstrated that *L. acidophilus* (ACD-DC 243and UNF La) and *L. casei* (CUP 121, and UNF LC) were resistant to vancomycin, ampecilline and erythromycin. Charteris *et al*. (2000) found that 7 of 37 *Lactobacillus* strains were resistance for antibiotics in presence of bile salt. However, Yazid *et al*. (2000) investigated the susceptibility of 18 bifidobacteria strains to 36 antibiotics and found that all tested strains were susceptible to several groups of antibiotics.

Table (6): Antibiotic resistance profiles of some microorganisms.

Antibiotics	Conc. µg/ml	Microorganisms					
		L. acidophilus	*L. casei*	*L. helveticus*	*B. bifidum*	*K. lactis*	*Sacch. cerevisiae*
Ampecilline	10	R	R	R	R	R	R
Chloramphincol	30	R	R	R	R	R	R
Erythromycin	15	R	R	R	R	R	R
Neomycin	30	R	R	R	R	S	S
Tetracyclin	30	R	R	R	R	R	R
Vancomycin	30	R	R	R	R	R	R

Results are expressed as R (resistant), S (susceptible).

7. Release of microencapsulated cells in simulated colonic solution:

An efficient release of viable and metabolically active cells in the intestine is one of the aims of microencapsulation. The release of cells from microcapsules in colon is essential for growth and colonization of probiotics; otherwise the microorganisms in the beads will be washed out from the body without exerting any beneficial effect.

Data obtained in Table (7) show that the released cell counts were between 3.60 and 3.90 log cfu/g on immediate exposure to the solution of simulated colonic pH from an initial count 8.20 and 8.45 logcfu/ml and as the exposure time increased to 90 min, the release of cells from their microcapsules increased significantly. On the other hand data obtained showed that there was no significant change in the number of viable cells released after 90 and those after 180 min exposure time. Similar observations were reported by Picot and Lacroix (2004) who found that there was a progressive release of viable cells from whey protein based microcapsules in simulated intestinal condition. The same trend was also observed by Mandal *et al*. (2006) who reported that the released cell counts of *L. casei* NCDC-298 were between 3.40 and 3.70 log cfu/g on immediate exposure to the solution of simulated colonic pH from an initial count 9.40 log cfu/g and the release of cells was increased with the increase of incubation time.

Table (7): Release of microencapsulated cells of some microorganisms in simulated colonic pH solution.

Strains	Initial Count	Exposure time (min) at 37˚C		
		0	90	180
	Log cfu/ml			
L. acidophilus La-5	8.30	3.80	8.25	8.26
L. casei - 01	8.40	3.90	8.30	8.36
L. helveticus Lh. B 02	8.45	3.60	8.35	8.40
B. bifidum Bb-12	8.36	3.80	8.30	8.35
K. lactis NRRL Y- 8279	8.28	3.60	8.00	8.15
Sacch. cerevisiae DSMZ 70 449	8.20	3.80	8.12	8.15

$LSD_{0.05}$ of experimental treatments = 0.62 (*P* <0.05)

8. Cell surface hydrophobicity:

Cell surface hydrophobicity is one of the physicochemical properties that facilitate the first contact between the microorganism and the host cells. The high cell surface hydrophobicity may favor the colonization of mucosal surfaces and play a role in the adhesion of bacteria to epithelial cells and extracellular matrix (ECM) proteins (Zareba *et al*., 1997).

Using an in vitro system, the hydrophobicity of the tested strains cell surface was determined photometrically using microbial adhesion to hydrocarbons (MATH) assay. Results of this assay showed considerable difference in the percentage of hydrophobicity values between different strains as shown in Table (8). It can be observed that *B. bifidum* Bb-12 exhibited the highest hydrophobicity value as it was 80% as compared to other strains. However, *L. acidophilus* La-5 and *L. casei* 01 revealed a

relatively hydrophobic nature of 68 and 52% respectively. On the other hand, *L. helveticus* Lh.B 02, *K. lactis* NRRL Y 8279 and *Sacch. cerevisiae* DSMZ 70449 characterized by low hydrophobicity of 35, 30 and 22% respectively. These results are almost in accordance with those reported by Schillinger *et al*. (2005) who found that *L. acidophilus* strains had the highest hydrophobicity as compared to *L. casei*.

Table (8): Cell surface hydrophobicity of some microorganisms.

Strains	Hydrophobicity of cell surface %
L. acidophilus La-5	68
L. casei - 01	52
L. *helveticus* Lh. B 02	35
B. *bifidum* Bb-12	80
K. lactis NRRL Y- 8279	30
Sacch. cerevisiae DSMZ 70 449	22

Conclusion

The results obtained showed that *K*. *lactis* NRRL Y-8279, *B. bifidum* Bb-12 and *L. casei* - 01 could survive well in GIT environment and reach the areas of beneficial activity when ingested. The final number of viable cells of these strains was still above the levels suggested to produce their claimed health benefits. The microencapsulation of microbial strains protected them against adverse effects of GIT environment and enhanced their survivability.

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تقييم الخصائص الداعمة للحيوية لبعض السلالات الميكروبية في بيئة مشابهه للقناة الهضمية.

فوزية حسن رجب عبدربه ، سامية محمود الديب ، سناء محمد بدران ، علاء محمد عبد الفتاح و فؤاد محمود فؤاد الشغبي.
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تناولت هذه الدراسة تقدير حيوية كل من *L. acidophilus* La-5, *L. casei* 01, *L. helveticus* Lh . B 02, *B. bifidum* Bb-12, *K. lactis* NRRL Y- 8279 , *Sacch. cerevisiae* DSMZ 70 449 في صورة حرة و مكبسلة في بيئة مشابة لتلك الظروف في القناة الهضمية وكانت أهم النتائج المتحصل عليها كالآتي: انخفضت أعداد الخلايا الحية معنوياً فيما عدا *B. bifidum, K. lactis* في الصورة المكبسلة مقارنة بالعدد المبدئي لها في وجود الليسوزيم بتركيز ١٠٠ ميكروجرام / مل. أنخفضت حيوية كل السلالات عند pH 1.5 عن هذه عند pH 3 خلال وقت التعرض لدرجة ٣٧°م / ١٨٠ق.أدت زيادة تركيز ملح الصفراء الي انخفاض مقاومة كل الميكروبات معنوياً مقارنة بأعدادها المبدئية خلال وقت التعرض لدرجة ٣٧°م / ١٨٠ق وبالنسبة لنشاط الأنزيم المحلل للصفراء أمكن الكشف عنه في كل من *B. bifidum, L. acidophilus* . انخفضت أيضاً حيوية كل السلالات مع زيادة تركيز الفينول ووقت التعرض. ولوحظ أيضاً مقاومة كل السلالات لكل المضادات الحيوية المستخدمة في هذه الدراسة فيما عدا الخمائر التي لوحظ حساسيتها للنيوميسين. أظهرت نتائج هذه الدراسة اختلافات واضحة بين السلالات المختلفة في قدرتها علي الالتصاق. بصفة عامة كان العدد النهائي للخلايا الحية أكبر من المستويات المقترحه للحصول على التأثير الصحي وعلى الجانب الآخر ساعدت الكبسلة فى حماية السلالات من التأثير المعاكس لبيئة القناة الهضمية وساعدت في تحسين حيوية هذه السلالات.