Effect of Encapsulation on some Probiotic Criteria

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Abstract: The present study was conducted to evaluate the ability of twelve non-encapsulated and encapsulated lactic acid and bifidobacteria strains to withstand environmental conditions similar to the human digestion tract. Selection criteria employed included the ability of these strains to survive at low pH and relatively high bile concentrations. Cholesterol assimilation and the effects of exposure to simulated gastric and intestinal juices were also investigated to explore the effect of encapsulation on health beneficial effect of the tested strains. The results obtained clearly declared that encapsulation effectively protected the microorganisms from the hostile environment and gastrointestinal tract, thus potentially preventing cell loss. The survival rate of encapsulated bacteria at pH 2.0 increased and attained a mean value of 58.9 % as compared with the corresponding value for non-encapsulated strains, being 46.9 %. Encapsulated cultures attained the highest tolerance % at different bile concentrations up to 1.0 %. Continuously, the survival percent of the double effects of pH and bile salt showed higher values and ranged from 34.15 % to 57.71 % for encapsulated bacteria, while free cells ranked lower figures varied from 17.15 % to 43.20 %. The assimilative reductions of cholesterol by non-encapsulated and encapsulated strains were clearly differed and varied from 32.6 % to 89.3 % and 27.9 % to 85.1 % respectively. The survival of encapsulated tested cultures in simulated gastric environment (SGJ) was noticeably better than those of non-encapsulated strains. In contrast, either free cells or encapsulated bacteria survival well in simulated intestinal juice (SIJ). [Journal of American Science 2010;6(10):810-819]. (ISSN: 1545-1003).

Keywords: encapsulated lactic acid; bifidobacteria; human digestion tract; simulated gastric environment (SGJ)

1. Introduction:

Probiotic bacteria are frequently used as the active ingredient in functional foods such as bio-yoghurts, dietary adjuncts and health-related products (Brassart and Schiffrin, 1997). The health benefits attributed to probiotic bacteria can be categorized as either nutritional benefits or therapeutic benefits. Nutritional benefits include: their role in enhancing the bio-availability of calcium, zinc, iron, manganese, copper and phosphorus (McDonough et al., 1983) and synthesis of vitamins (Deeth and Tamime, 1981). While the therapeutic benefits of these bacteria including antimicrobial activity, ability to assimilate cholesterol, improved lactose intolerance and anti-carcinogenic activity (Chou and Weimer, 1999).

The majority of probiotic bacteria belong to two bacterial genera: i.e. Lactobacillus and Bifidobacterium. A stringent selection criteria for identification of probiotic strains is required in order to achieve consistent and positive probiotic effects. Most recently Collins et al. (1998) have compiled a list of 12 important criteria for selecting a potential probiotic strains. Essentially, these criteria suggest the selected strains must be safe, viable and metabolically active within the gastrointestinal tract in order to exert a beneficial impact on the host. Therefore, International Dairy Federation (IDF) has suggested that a minimum of 10^7 probiotic bacteria cells should be alive at the time of consumption per gram of the product (Homayouni et al. 2007c). In order to improve the survival of probiotic bacteria many attempts have been carried out (Modler et al., 1990; Mituoka, 1992; Ravula and Shah, 1998; Chou and Weimer, 1999 and Sultana et al., 2000). However, these trails had only a limited success.

Therefore, encapsulation technique have been investigated as a physical protection of probiotics for improving its viability and survival (Sultana et al., 2000; Chandramouli et al., 2004 and Picot and Lacroix, 2004).

Thus, the objective of this study was to screen the tested strains for functional characteristics of probiotics in order to gain more information concerning their ability to tolerate acid, bile, assimilate cholesterol and resist the digestion in the intestinal tract. In addition, the main target was to evaluate the feasibility of encapsulation to improve the probiotic survival of encapsulated cultures under the hostile environment and gastrointestinal tract.

2. Materials and methods

Bacterial strains:

Eight strains of lactic acid bacteria and four strains of bifidobacteria were obtained from different cultures collections which summarized in Table 1.

Lactic acid cultures were maintained and subcultured in MRS broth using a 1 % inoculum and

18 to 20 hr. of incubation at 37ºC. Bifidobacteria were enumerated according to **Dave and Shah (1996)** using modified MRS agar medium (m-MRS), supplemented with 0.05 % L-cysteine HCL and 0.3 % lithium chloride and cultures were propagated at 37ºC for 24 h.

Table (1): Source of various bacterial strains used in the present investigation:

Bacterial strains	Source
Lb. delbrukii subsp bulgaricus EMCC 11102	Cairo MIRCEN
Lb. johnsonii ATCC 33200	ATCC
Lb. casei EMCC 11093	Cairo MIRCEN
Lb. acidophilus ATCC 4356	ATCC
Lb. acidophilus ATCC 20552	ATCC
Lc. lactis subsp. cremoris ATCC 19257	Dairy Department, Minia University
Lc. lactis subsp. lactis EMCC 11552	Dairy Department, Minia University
Str. thermophilus EMCC11044	Cairo MIRCEN
Bif. bifidum 2203	Cairo MIRCEN
Bif. bifidum ATCC 15696	Cairo MIRCEN
Bif. angulatum 2338	Cairo MIRCEN
Bif. longum ATCC 2259	ATCC

Abbreviations: EMCC, Egyption Microbial Culture Collection.
ATCC, American Type Culture Collection.
Cairo MIRCEN, Cairo Microbiological Resources Center, Faculty of Agriculture, Ain Shams University.

Acid tolerance:

All tested cultures were evaluated for their ability to grow in low pH 2 and 3 according to Pereira and Gibson (2002) with some modifications: Cultures were grown to stationary phase (16 hr.) in either MRS or mMRS broth to an optical density (OD_{650}) of 1.2 – 1.3 with fresh media. Tested cultures were inoculated (10 % vol./vol.) into MRS or mMRS broth previously adjusted to pH 2 and 3 with HCl. The mixtures were incubated at 37°C for 90 minutes. Cultures were monitored for growth spectrophotometrically at 650_{nm}. at 0, 30, 60 and 90 minutes. The experiments were repeated three times.

Bile tolerance:

Overnight cultures were inoculated (1 % vol./vol.) into mMRS broth and m-MRS broth containing 0.3, 0.5, 0.7 and 1.0 % (wt/vol.) oxgall and incubated at 37°C for 12 hr. Cultures were monitored for growth spectrophotometrically at 650_{nm}. Comparison of cultures was based on their growth rates in each broth. The experiments were repeated three times in triplicate (Pereira and Gibson, 2002).

Bile and Acid tolerance:

Active culture were inoculated (10 % vol./vol.) into either MRS or m-MRS broth previously adjusted to pH 3.0 with HCl and incubated at 37°C for 24 hours. Pre-exposed cells were resuspended in MRS or m-MRS broth containing bile salt at final concentration of 0.3 % (wt/vol) oxgall. Tested culture was incubated again at 37°C for 12 hr. and the growth was monitored spectrophotometrically at 650_{nm}. (Prasad et al., 1998).

Assimilation of cholesterol:

The ability of tested cultures to assimilate cholesterol was determined according to Danielson et al. (1989) with some modifications as described by Pereira and Gibson (2002). Bile concentrations of 0.3 % (w/v) oxgall was used to mimic approximate levels in the intestinal tract (Sjovall, 1959). The cholesterol in the spent broth was first extracted by the procedure described by Gilliland et al. (1985). While, the total cholesterol concentration of the evaporated residues was then determined by the enzymatic assay described by Sale et al. (1984).

Preparation of simulated gastric juice (SGJ):

The SGJ was prepared with pH adjusted MRS broth to 1.4 or 7.0 (control) with 5 mol/L HCl or 1 mol/L NaOH sterilized solution. Suspending pepsin (1000 unit/ml) in MRS was sterile-filtered through a membrane filter and 0.1 ml of suspending pepsin was inoculated to 9.9 ml of SGJ (Kim et al. 2008).

Preparation of simulated small intestinal juice (SSIJ):

The SSIJ was prepared by dissolving pancreatin (Sigma) from porcine pancreas (1g/L) in sterile saline (5g/L) according to Charteris et al. (1998). Subsequently, the pH of the pancreatic juice was adjusted to pH 8 with 0.1 M NaOH. A 0.1 ml

pancreatic solution was added to 9.9 ml of MRS broth and sterilized at 121°C for 15 min.

Survival to simulated gastric juice (SGJ) or simulated small intestinal juice (SSIJ):

Each tested culture was incubated in MRS broth at 30°C for 24 h. A one ml aliquot of the tested culture was centrifuged at 5000×g for 10 min at 4°C and washed three times in sterile PBS. The washed cells were resuspended in PBS.

To assay the SGJ or SSIJ tolerance, about 0.2 ml of each washed cell suspension mixed with 1 ml of gastric or intestinal juice, after brief vortexing, the mixture was incubated at 37°C. When assaying gastric tolerance, aliquots of 0.1 ml was removed after 5, 40 and 180 min and the growth was monitored spectrophotometrically at 650_{nm}. For assaying small intestinal juice tolerance, the sampling times were 5, 240 and 360 min. The experiment was repeated twice, and each reading represents the mean of three observations (Guerra et al. 2007).

Encapsulation procedure:

All glasswares and solutions used in the protocols were sterilized at 121ºC for 15 min. Alginate beads were produced a modified encapsulation method originally reported by Sheu and Marshall (1993) and Sultana et al. (2000). A 2 % alginate mixture was prepared containing 2 % Hi-maize resistant starch and 0.1 % culture. The mixture was dropped into oil, containing Tween 80 (0.02 %). After the dropping was completed, the mixture was stirred vigorously till it was emulsified and appeared creamy. A solution of 0.1 M calcium chloride was then added quickly along the side of the beaker, the phase separation of oil /water emulsion occurred. The mixture was allowed to stand for 30 min for the calcium-alginate beads to separate and settle at the bottom of the calcium chloride layer. The oil layer was drained and beads were collected by low speed centrifugation (350×g, 15 min), washed once with 0.9 % saline containing 5 % glycerol, and stored at 4ºC.

3. Results and Discussion:

Bacteria used as probiotic adjuncts are commonly delivered in a food system and therefore begin their journey to the lower intestinal tract via the mouth, the time from entrance to release from the stomach about 90 min. (Berrada et al., 1991). Although cellular stress begins in the stomach, which has pH as low as 1.5 (Lankaputhra and Shah, 1995), in most in vitro assays pH 3.0 has been preferred (Garriga et al., 1998 and Suskovic et al., 1997). Thus, preliminary screening of all tested cultures for tolerance to low pH 2.0 and 3.0 was conducted.

Moreover, several studies indicate that the bacteria may not survive in sufficient numbers during their passage through the gastrointestinal tract (Dave and Shah, 1996 and Hamilton-Miller et al., 1999). Providing probiotic living cells with a physical barrier against adverse environmental conditions is therefore an approach currently considerable interest (Kailasapathy, 2002). Thus, encapsulation technique have been applied to increase the survival and delivery of bacterial cultures (Sultana et al., 2000).

Results presented in Table (2) indicated that all non- encapsulated tested strains were strongly affected at pH 2.0, while relatively moderate tolerance obtained at pH 3.0. In this respect, Maffei and Nobrega (1975) stated that the bactericidal effect of acid is evident at pH values below 2.5. Also, data obtained revealed that growth inhibition for non-encapsulated tested strains were gradually increased with prolongation of incubation time till 90 min at either pH 2.0 or pH 3.0. These results are generally in harmony with those reported by Shah and Jelen (1990), they mentioned that the survival of the lactic acid bacteria were reduced as incubation time increased and pH decreased.

As shown from Table 2, encapsulated bacteria survived well in low pH 2.0 compared to non-encapsulated free bacterial cells. However, the survival rate of encapsulated bacteria increased and varied from 42.64 to 72.93 % with a mean value of 58.9 %, as compared with the corresponding value for non-encapsulated strains ranged from 34.03 to 68.10 % with a mean value, actually 46.9 %. As expected, at pH 3.0, better protection of bacterial cells was achieved. However, the same trend of result was previously reported by Sultana et al. (2000) and Chandramouli et al. (2004).

Although the bile concentration of the human gastrointestinal tract varies, the mean intestinal bile concentration is believed to be 0.3 % w/v. (Suskovic et al., 1997 and Garriga et al., 1998). Therefore, four different bile concentrations were used 0.3, 0.5, 0.7 and 1.0 % (w/v) oxgall, to mimic approximate levels in the intestinal tract.

From the data presented in Table 3, it could be pointed out that tolerance to bile varied among the tested strains and bile concentrations. In general, encapsulated strains exhibited more bile resistance compared to free cells under similar conditions. Continuously, encapsulated cultures attained the highest tolerance percent at bile concentrations up to 1.0 % (w/v), being 90.26, 87.75, 86.36 and 84.38 % at 0.3, 0.5, 0.7 and 1.0 % (w/v), respectively. Similar improvements in survival have previously reported for lactobacilli and bifidobacteria encapsulated strains by Chandramouli et al. (2004) and Kim et al. (2008).

Obviously, inability of non-encapsulated tested strains to survive the double effects of pH and bile salt was clearly detected in the free cells where their survival rate declined and varied from 17.15 % to 43.20 % after 12 h of incubation at 0.3 % (w/v) oxgall. On the other hand, encapsulated bacteria survived better under the same conditions and their survival percent showed higher value and ranged from 34.15 to 57.71 % (Table 4).

Increased serum cholesterol correlate highly with the incidence of coronary heart disease (Kern, 1991). However, dietary adjustments is one way to decrease serum cholesterol, thereby reducing the risk of coronary heart disease.

In our study, the ability of non-encapsulated and encapsulated strains to assimilate cholesterol was carried out and the data obtained presented in Table 5. Viewing these results, it might be deduced that all tested cultures grew well in the presence of oxgall and reduced cholesterol concentration in the culture broth.

Table (2): Effect of low pH on viability and survival of non-encapsulated and encapsulated strains.

Tested strains	pH	Non-encapsulated					Encapsulated				
		Incubation time / min. decrease of OD_{650}									
		Zero	**30**	**60**	**90**	**Survival%**	**Zero**	**30**	**60**	**90**	**Survival%**
***L. bulgaricus* EMCC 11102**	2	1.252	0.820	0.534	0.426	34.03	1.407	1.010	0.715	0.600	42.64
	3	1.255	0.936	0.74	0.544	43.35	1.410	1.131	0.925	0.722	51.21
***L. johnsonii* ATCC 33200**	2	1.260	0.884	0.834	0.493	39.13	1.415	1.074	1.015	0.667	47.14
	3	1.265	1.067	0.901	0.670	52.96	1.420	1.262	1.086	0.848	59.72
***L. casei* EMCC 11093**	2	1.252	0.955	0.638	0.448	35.78	1.407	1.145	0.820	0.622	44.21
	3	1.254	0.985	0.718	0.486	38.76	1.409	1.180	0.903	0.664	47.13
***L. acidophilus* ATCC 4356**	2	1.253	0.899	0.592	0.494	39.43	1.408	1.089	0.773	0.668	47.44
	3	1.254	0.945	0.779	0.667	53.19	1.409	1.140	0.964	0.845	59.97
***L. acidophilus* ATCC 20552**	2	1.260	0.884	0.740	0.613	48.65	1.415	1.074	0.921	0.787	55.62
	3	1.263	0.954	0.929	0.807	63.90	1.418	1.149	1.114	0.985	69.46
Lc. Lactis subsp. cremoris	2	1.252	1.054	0.866	0.646	51.60	1.407	1.244	1.047	0.820	58.28
	3	1.254	1.157	0.927	0.737	58.77	1.409	1.352	1.112	0.915	64.94
Lc. Lactis subsp. lactis	2	1.256	0.889	0.759	0.581	46.26	1.411	1.079	0.940	0.755	53.51
	3	1.259	0.983	0.915	0.638	50.68	1.414	1.178	1.100	0.816	57.71
***Str. thermophilus* EMCC11044**	2	1.260	1.073	1.015	0.858	68.10	1.415	1.263	1.196	1.032	72.93
	3	1.264	1.245	1.135	0.972	76.90	1.419	1.401	1.320	1.150	81.04
***Bif. bifidum* 2203**	2	1.258	1.024	0.813	0.645	51.27	1.413	1.214	0.994	0.819	57.96
	3	1.262	1.109	0.951	0.827	65.53	1.417	1.304	1.135	1.005	70.92
Bif. bifidum ATCC 15696	2	1.257	0.971	0.789	0.609	48.45	1.412	1.161	0.969	0.783	55.45
	3	1.259	1.071	0.958	0.794	63.07	1.414	1.266	1.140	0.972	68.74
***Bif.angulatum* 2338**	2	1.255	1.109	0.878	0.691	55.06	1.410	1.299	1.058	0.865	61.35
	3	1.260	1.165	0.980	0.844	66.98	1.415	1.360	1.164	1.022	72.23
***Bif. longum* ATCC 2259**	2	1.251	0.960	0.769	0.561	44.84	1.406	1.150	0.950	0.735	52.28
	3	1.260	1.096	0.931	0.646	51.27	1.415	1.291	1.117	0.824	58.23

Table (3): Survival of non-encapsulated and encapsulated tested cultures after exposure to different concentrations of bile salt.

Tested strains	Bile %	Non-encapsulated			Encapsulated		
		Incubation time / hr. decrease of OD_{650}					
		zero	12 hr.	Survival%	zero	12 hr.	Survival%
***L. bulgaricus* EMCC 11102**	0.3	1.132	0.812	71.73	1.405	1.265	90.04
	0.5	1.134	0.755	66.58	1.407	1.222	86.85
	0.7	1.134	0.703	61.99	1.407	1.206	85.71
	1	1.136	0.645	56.78	1.410	1.168	82.84
***L. johnsonii* ATCC 33200**	0.3	1.132	0.801	70.76	1.405	1.264	89.96
	0.5	1.133	0.743	65.58	1.406	1.220	86.77
	0.7	1.134	0.689	60.76	1.407	1.204	85.57
	1	1.134	0.628	55.38	1.408	1.173	83.31
***L. casei* EMCC 11093**	0.3	1.132	0.942	83.22	1.405	1.266	90.11
	0.5	1.134	0.831	73.28	1.407	1.225	87.06
	0.7	1.134	0.815	71.87	1.407	1.208	85.86
	1	1.136	0.646	56.87	1.409	1.179	83.68
***L. acidophilus* ATCC 4356**	0.3	1.133	0.802	70.79	1.407	1.267	90.05
	0.5	1.135	0.738	65.02	1.409	1.231	87.37
	0.7	1.136	0.668	58.80	1.409	1.211	85.95
	1	1.136	0.594	52.29	1.410	1.179	83.62
***L. acidophilus* ATCC 20552**	0.3	1.132	0.932	82.33	1.404	1.261	89.81
	0.5	1.134	0.852	75.13	1.407	1.221	86.78
	0.7	1.135	0.719	63.35	1.408	1.214	86.22
	1	1.137	0.658	57.87	1.409	1.182	83.89
Lc. lactis subsp. cremoris	0.3	1.133	0.722	63.72	1.404	1.263	89.96
	0.5	1.135	0.609	53.66	1.405	1.218	86.69
	0.7	1.136	0.532	46.83	1.406	1.209	85.99
	1	1.138	0.376	33.04	1.408	1.188	84.38
Lc. lactis subsp. lactis	0.3	1.133	0.924	81.55	1.407	1.270	90.26
	0.5	1.135	0.788	69.43	1.409	1.228	87.15
	0.7	1.136	0.672	59.15	1.409	1.207	85.66
	1	1.136	0.602	52.99	1.411	1.174	83.20
Str. thermophilus	0.3	1.130	0.744	65.84	1.402	1.259	89.80
	0.5	1.132	0.623	55.04	1.405	1.224	87.12
	0.7	1.133	0.511	45.10	1.407	1.205	85.64
	1	1.135	0.444	39.12	1.409	1.186	84.17
***Bif. bifidum* 2203**	0.3	1.136	0.921	81.07	1.406	1.266	90.04
	0.5	1.138	0.863	75.83	1.406	1.218	86.63
	0.7	1.138	0.811	71.27	1.408	1.213	86.15
	1	1.139	0.723	63.48	1.411	1.184	83.91
Bif. bifidum ATCC 15696	0.3	1.132	0.982	86.75	1.403	1.258	89.67
	0.5	1.135	0.917	80.79	1.405	1.214	86.41
	0.7	1.137	0.862	75.81	1.407	1.208	85.86
	1	1.138	0.800	70.30	1.409	1.185	84.10
***Bif. angulatum* 2338**	0.3	1.131	0.969	85.68	1.403	1.255	89.45
	0.5	1.133	0.902	79.61	1.406	1.226	87.20
	0.7	1.134	0.854	75.31	1.408	1.216	86.36
	1	1.135	0.795	70.04	1.409	1.182	83.89
***Bif. longum* ATCC 2259**	0.3	1.134	0.994	87.65	1.404	1.248	88.89
	0.5	1.136	0.923	81.25	1.404	1.232	87.75
	0.7	1.137	0.875	76.96	1.407	1.214	86.28
	1	1.137	0.821	72.21	1.410	1.177	83.48

Table (4): Effect of low pH and bile salt 0.3 % oxgall on non-encapsulated and encapsulated tested strains.

Tested strains	Non-encapsulated				Encapsulated			
	Zero/ Survival %	OD_{650} after incubation time			Zero/ Survival %	OD_{650} after incubation time		
		24 h	6 h	12 h		24 h	6 h	12 h
***L. bulgaricus* EMCC 11102**	1.255	0.360	0.344	0.254	1.412	0.637	0.605	0.522
	Survival%	28.69	27.41	20.24	Survival%	45.11	42.85	36.97
***L. johnsonii* ATCC 33200**	1.265	0.445	0.398	0.362	1.410	0.722	0.675	0.630
	Survival%	35.18	31.46	28.62	Survival%	51.21	47.87	44.68
***L. casei* EMCC 11093**	1.254	0.311	0.284	0.215	1.420	0.588	0.539	0.485
	Survival%	24.80	22.65	17.15	Survival%	41.41	37.96	34.15
***L. acidophilus* ATCC 4356**	1.254	0.482	0.362	0.275	1.409	0.759	0.636	0.535
	Survival%	38.44	28.87	21.93	Survival%	53.87	45.14	37.97
***L. acidophilus* ATCC 20552**	1.263	0.618	0.544	0.462	1.409	0.891	0.811	0.733
	Survival%	48.93	43.07	36.58	Survival%	63.24	57.56	52.02
Lc. lactis subsp. cremoris	1.254	0.555	0.435	0.382	1.418	0.832	0.708	0.644
	Survival%	44.26	34.69	30.46	Survival%	58.67	49.93	45.42
Lc. lactis subsp. lacti	1.259	0.471	0.359	0.273	1.409	0.748	0.633	0.551
	Survival%	37.41	28.51	21.68	Survival%	53.09	44.93	39.11
***Str.thermophilus* EMCC11044**	1.264	0.798	0.642	0.546	1.414	1.072	0.912	0.816
	Survival%	63.13	50.79	43.20	Survival%	75.81	64.50	57.71
***Bif. bifidum* 2203**	1.262	0.657	0.536	0.421	1.419	0.931	0.807	0.691
	Survival%	52.06	42.47	33.36	Survival%	65.61	56.87	48.70
***Bif. bifidum* ATCC 15696**	1.259	0.602	0.482	0.376	1.417	0.873	0.753	0.648
	Survival%	47.82	38.28	29.86	Survival%	61.61	53.14	45.73
***Bif. angulatum* 2338**	1.260	0.673	0.545	0.461	1.414	0.943	0.817	0.703
	Survival%	53.41	43.25	36.59	Survival%	66.69	57.78	49.72
***Bif. longum* ATCC 2259**	1.260	0.485	0.381	0.301	1.415	0.758	0.646	0.571
	Survival%	38.49	30.24	23.89	Survival%	53.57	45.65	40.35

It is clear that the cell dry weight was approximately the same with non-encapsulated and encapsulated cells, varied from 1.0 to 2.3 and 0.9 to 2.4 g/L, respectively. Moreover, the non-encapsulated strains ranked higher cholesterol uptake percent, varied from 36.2 to 52.5 %, while the corresponding figures in the encapsulated cells ranged from 27.9 to 42.6 %. Additionally, from obtained results it could be observed that the amount of cholesterol removed from the broth was higher actually varied from 32.6 to 89.3 mg/L for cell free strains, while the effect of encapsulated cultures was lower, actually ranged from 27.9 to 85.1 mg/L. In this respect, Kim et al. (2008) stated that encapsulation reduce the ability of lactic acid bacteria to assimilate cholesterol. The same phenomenon was also detected by Khater (2009).

Survival of bacteria in human gastric juice is of a more accurate indication of the ability of strains to survive transit through the stomach (Mathara et al., 2008). Therefore, the tolerance of the tested culture to simulated gastric juice was determined and results obtained presented in Table 6. Generally, all tested strains showed slight/moderate decreases varied from 0.53 % to 10.40 % through 180 min of exposure. However, the survival of encapsulated tested cultures in gastric environment was noticeably better than those of non-encapsulated strains. The survivors encapsulated strains in SGJ maintained above 92 % after 3 hours of incubation. In contrast, free cells were more sensitive to gastric conditions and suffered

a reduction in their survival rate which actually varied from 89.60 to 92.93 %. Similar trend of result was previously recorded by Chan and Zhang (2005) and Chandramouli et al. (2004).

In order to exert positive health effects, probiotic bacteria resist the stressful conditions of the stomach and upper intestine that contain bile (Chou and Weimer, 1999). Thus, survivals of tested cultures were monitored up to 360 min after exposure to SIJ.

It was obvious, as the data in Table 7, that either non-encapsulated or encapsulated bacteria survived well in simulated intestinal juice (SIJ). In this connection, Charteris et al. (1998) reported that the majority of probiotic strains were intrinsically resistant to simulated pancreatic juice and showed no reduction in viability up to 4 hours. In addition, Buck and Gilliland (1994) and Sultana et al. (2000) stated that the encapsulated bacteria did not demonstrate a significant increase in survival when subjected to in vitro SIJ. Also, it was of interest to notice that viability of *L. acidophilus ATCC 4356* in SIJ remained constant after 6 h. of incubation wither encapsulated or not.

Generally, from the forgoing results, it could be concluded that tested cultures selected for this study differed widely in their probiotic characteristics. On the other hand, the results obtained revealed that encapsulation can increase the survival rate of probiotic bacteria in low pH and different concentrations of bile salt. In contrast, our study showed that encapsulated gave little injury to tested cultures in assimilation of cholesterol. While the process enhanced the survival rate of the tested cultures in the simulated gastric environment.

However, future studies need to be carried out in order to monitor the effect of encapsulation on bacteria in the gut, using animal models, as well as studying other parameters.

Table (5): Assimilation of cholesterol by non-encapsulated and encapsulated lactic acid and bifidobacteria strains.

Tested strains	DCW g/L		Residual cholesterol mg/L		Cholesterol uptake (%cholesterol g dry weight)	
	Non	En.	Non	En.	Non	En.
***L. bulgaricus* EMCC 11102**	2.1	1.9	21.84	20.20	42.12	37.32
***L. johnsonii* ATCC 33200**	2.0	1.7	10.70	14.90	52.50	42.60
***L. casei* EMCC 11093**	1.9	2.3	11.30	16.60	46.70	36.10
***L. acidophilus* ATCC 4356**	2.2	2.4	11.70	16.90	40.10	34.60
***L. acidophilus* ATCC 20552**	2.0	2.3	18.90	25.30	40.60	32.50
Lc. lactis subsp. cremoris	2.1	1.8	20.51	33.22	44.14	37.13
Lc. lactis subsp. lacti	1.9	2.3	11.50	16.50	46.60	36.30
***Str. thermophilus* EMCC11044**	1.8	2.1	11.90	17.20	48.90	39.40
***Bif. bifidum* 2203**	1.8	2.1	18.70	25.30	42.80	35.60
***Bif. bifidum* ATCC 15696**	2.3	1.9	21.82	25.31	46.02	39.31
***Bif. angulatum* 2338**	1.0	0.9	67.40	72.10	36.20	27.9
***Bif. longum* ATCC 2259**	1.9	2.2	18.60	25.20	42.80	34.00

DCW= Dry cell weight Non= non-encapsulated En.= encapsulated

Table (6): Effect of simulated gastric juice (SGJ) on viability of lactic acid bacteria and bifidobacteria strains.

Tested strains	Non-encapsulated and					Encapsulated				
	Incubation time (min.)									
	Zero	5	40	180	Survival %	Zero	5	40	180	Survival %
	OD_{650}	OD650	OD650	OD650		OD650	OD650	OD650	OD650	
***L. delbruckii subsp. bulgaricus* EMCC 11102**	1.320	1.310	1.280	1.220	92.93	1.430	1.430	1.410	1.360	95.03
***L. johnsonii* ATCC 33200**	1.300	1.280	1.250	1.180	90.79	1.420	1.400	1.380	1.320	93.37
***L. casei* EMCC 11093**	1.290	1.280	1.250	1.180	91.16	1.400	1.400	1.390	1.330	94.80
***L. acidophilus* ATCC 4356**	1.310	1.290	1.250	1.170	89.60	1.420	1.410	1.380	1.280	90.15
***L. acidophilus* ATCC 20552**	1.300	1.270	1.270	1.210	92.84	1.410	1.380	1.380	1.340	94.62
***Lc. lactis subsp. cremoris* ATCC 19257**	1.310	1.300	1.280	1.220	92.84	1.430	1.420	1.400	1.370	95.66
***Lc. lactis subsp. lactis* EMCC 11552**	1.290	1.270	1.240	1.170	90.18	1.410	1.390	1.370	1.310	92.82
***Str. thermophilus* EMCC11044**	1.310	1.300	1.280	1.220	92.68	1.430	1.420	1.410	1.350	94.88
***Bif. bifidum* 2303**	1.320	1.310	1.280	1.220	92.05	1.430	1.430	1.400	1.360	94.56
***Bif. bifidum* ATCC 15696**	1.300	1.280	1.250	1.180	90.32	1.420	1.400	1.380	1.310	92.44
***Bif. angulatum* 2238**	1.330	1.320	1.280	1.220	91.30	1.450	1.430	1.410	1.350	93.09
***Bif. longum* ATCC 2259**	1.320	1.300	1.270	1.200	90.92	1.450	1.420	1.390	1.350	92.64

Table (7): Effect of simulated intestinal juice (SIJ) on viability of lactic acid bacteria and bifidobacteria strains.

Tested strains	Non-encapsulated and					Encapsulated				
	Incubation time (min.)									
	Zero	5	240	360	Survival %	Zero	5	240	360	Survival %
	OD_{650}	OD650	OD650	OD650		OD650	OD650	OD650	OD650	
***L. delbruckii subsp. bulgaricus* EMCC 11102**	1.313	1.313	1.307	1.294	98.55	1.428	1.428	1.426	1.417	99.23
***L. johnsonii* ATCC 33200**	1.302	1.298	1.293	1.293	99.31	1.416	1.414	1.412	1.411	99.65
***L. casei* EMCC 11093**	1.288	1.283	1.279	1.270	98.60	1.403	1.399	1.396	1.389	99.00
***L. acidophilus* ATCC 4356**	1.305	1.305	1.305	1.305	100.00	1.419	1.419	1.419	1.419	100.00
***L. acidophilus* ATCC 20552**	1.297	1.292	1.284	1.283	98.92	1.411	1.411	1.402	1.397	99.01
***Lc. lactis subsp. cremoris* ATCC 19257**	1.310	1.307	1.296	1.285	98.09	1.425	1.422	1.413	1.401	98.32
***Lc. lactis subsp. lactis* EMCC 1552**	1.293	1.293	1.287	1.274	98.53	1.407	1.407	1.404	1.404	99.79
***Str. thermophilus* EMCC11044**	1.308	1.307	1.304	1.298	99.24	1.424	1.420	1.420	1.414	99.30
***Bif. bifidum* 2203**	1.319	1.319	1.317	1.313	99.55	1.433	1.429	1.428	1.428	99.65
***Bif. bifidum* ATCC 15696**	1.298	1.294	1.286	1.275	98.23	1.412	1.409	1.403	1.394	98.73
***Bif. angulatum* 2338**	1.331	1.328	1.320	1.316	98.87	1.445	1.444	1.437	1.424	98.55
***Bif. longum* ATCC 2259**	1.320	1.320	1.316	1.309	99.17	1.434	1.430	1.432	1.426	99.44

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8/22/2010