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RESEARCH ARTICLE

MICROENCAPSULATION OF LACTOBACILLUS ACIDOPHILUS FOR ITS SURVIVAL UNDER HIGH ACID AND HIGH BILE SALT CONCENTRATIONS

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ABSTRACT

Probiotics are live microorganisms which when consumed in sufficient amounts are beneficial for human health. *Lactobacillus acidophilus* is a type of beneficial bacteria that has been studied extensively, for its beneficial properties. The survival and viability of probiotic bacteria is often low under simulated conditions of gastrointestinal tract which increases the chances of potential pathogens to dominate and cause infection. Among several methods that increase the survival of probiotics, Encapsulation serves the most rational technique. Hence this investigation reports the effect of microencapsulation using sodium alginate and starch on the tolerance or survival of probiotic *Lactobacillus acidophilus* to selected processing conditions and stimulated gastrointestinal environments. Microencapsulation provided better protection at stimulated conditions of gastric pH (1.0 and 2.0) and at high bile salt concentrations (1.0% and 2.0%). The release of the microencapsulated organisms at pH required 3 h. These studies demonstrated that microencapsulation of probiotic L. *acidophilus* in sodium alginate is an effective technique of protection against extreme processing conditions and under stimulated gastrointestinal environment.

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INTRODUCTION

Probiotics are live microorganisms and when consumed in adequate amounts they are beneficial for health (FAO/WHO 2001). They can be used as complementary and alternative medicine (CAM). *Lactobacillus acidophilus* help the system by replacing the friendly bacteria destroyed during antibiotic therapy. For their potential effects their viability must be maintained throughout storage, products shelf-life and they must survive the gut environment (Kailasapathy and Chin, 2000). Several reports have shown that survival and viability of probiotic bacteria is often low under simulated conditions of gastrointestinal tract. Once Probiotic organisms have been stripped off the walls of the intestine all pathogens tend to proliferate which leads to infection (Hill, 1995). This coincides with the reduction in no. of *Lactobacillus* sp (Lidbeck *et al*., 1988) There are several methods that increase the survival of probiotics viz. supplementation with growth promoters or casein hydrolysate, fructose, acetate addition enhanced the growth of L. *acidophilus* (Marshall, 1991). Many encapsulation strategies have been examined to increase the survival of these microorganisms by several workers (Rao *et al*., 1989; Kebary *et al.,* 1998; Khalil Mansour, 1998). Encapsulation of *L.acidophilus* means covering of bacteria with comparatively inert material towards different environment conditions. Encapsulation techniques include Physical methods eg: Spray drying, Spray chilling and cooling (Risch, 1995) and chemical methods like Entrapment of microorganisms in some gel matrix (Alginate and Starch). Influenced by the work of Shridhar (2003), effort in present study was to investigate the effect of encapsulation on the survival of *L. acidophilus* under stimulated gastric conditions.

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MATERIALS AND METHODS

Maintenance of Culture

Bacterial culture *Lactobacillus acidophilus* (NCIM 2902) was procured from NCL, Pune- Maharashtra. Culture was maintained on Modified MRS agar tubes. Loopful of stock culture was inoculated in 5 ml sterile MRS broth tubes and was incubated at 37° C for 24hrs. 6 hrs old culture was used for experimental purpose.

Effect of pH and Bile salt concentration

Tubes were divided in two sets 1 and 2 for the study of organism with and without encapsulation at different pH and bile concentration.

Each set further contain two subsets, a & b, Where,

a- 5 tubes with pH adjusted at 2, 3, 4, 5, 6.5 with 1% bile salts.
b- 5 tubes with pH adjusted at 2, 3, 4, 5, and 6.5 with 2% bile salts.

The control was kept at pH 6.8 with 1% and 2 % bile salts concentration for set a and set b respectively. Using micropipette, 1ml of 6-8 hrs old broth culture was transferred to the tubes of set 1. 1 µl of the sample from each tube, was plated on selective media (MRS agar), by spread plate method. The inoculated plates were then incubated at 37° C for 48 hrs and then colonies were counted using Quebec's colony counter and recoded as cfu/ml. The tubes were incubated for 3 hrs at 37° C and then 1µl sample from each tube was further spread on the plates. Cell count was recorded after 48 hrs incubation.

Encapsulation of *Lactobacillus acidophilus*

For encapsulation Sodium alginate (1gm/50ml) and Hi resistant starch (1gm/50ml) was homogenized and 5 ml of culture was added to this 100 ml of solution. This solution was filled in 1 ml disposable syringe and added drop wise in 0.1 M $Cacl_2$. Beads were formed and were kept in refrigerator overnight for hardening.

Effect of pH and Bile salt concentration

The encapsulated organism i.e beads were used for inoculation of set 2. Tubes were inoculated with 5 beads each, which is equivalent to 1 ml broth culture. Thus, number of cells inoculated can be obtained from the data collected from set 1.The tubes were incubated at 37° C for 3 hrs. The beads were crushed after 3 hrs incubation, within the broth using glass rod to homogenize the suspension and then transferred on MRS Agar plates, each 1 µl. The plates were incubated for 48 hrs at 37° C and then observe for colony counting using Quebec's colony counter. The number of colonies were counted and recorded as cfu/ml

RESULTS AND DISCUSSION

Lactobacillus acidophilus and *B. bifidum* are natural habitants of intestines of humans and animals as well. The stomach and the surroundings of gastrointestinal tract have highest acidity and bile salt concentrations. *Lactobacillus acidophilus* must be able to survive these adverse conditions in order to colonize in the gut. Survival of *L. acidophilus* and *B. bifidum* depends on the pH of the environment; low pH decreases their survival. Viability and activity of the bacteria are important consideration as the bacteria have to survive in food during transit through acidic condition in the stomach and bile salt in small intestine (Playne, 1993). In current investigation effect of pH and 1%, 2 % bile salt concentration was studied. It was clear that the free *Lactobacillus acidophilus* could tolerate pH 3.0 up to 3 hrs of incubation at different bile salts concentration 100% reduction at pH 2 (Table 1, 2) (Figure 1).The result revealed that the organism tolerate pH 3.0 up to 3 hrs of incubation at different bile salts concentration. Thus survival is further studied with encapsulation. Encapsulation studies (Table 3, 4) (Figure 2, 3) showed that the survival of *Lactobacillus acidophilus* under gastric conditions has been greatly enhanced by 9.52% in 2 % bile salt concentration and 7.14% at 1% bile concentration. Encapsulation of organism enhanced survival period at low pH up to pH 2 considerably at different bile salts concentration.

Table 1. Survival of *L.acidophilus* at different pH with 1% bile salts Concentrations (x 10^8 cfu/ml)

pH	Incubation Period (hrs)	Population	% Reduction
2.0	0	4.2	
	3	-	100
3.0	0	4.2	
	3	1.2	71.42
4.0	0	4.2	
	3	3.2	23.80
5.0	0	4.2	
	3	3.5	16.66
6.0	0	4.2	
	3	4.1	02.38
6.8	0	4.2	
(control)	3	4.2	-

Table 2. Survival of *L.acidophilus* at different pH with 2% bile salts Concentrations (x 10^8 cfu/ml)

Ph	Incubation Period (hrs)	Population	% Reduction
2.0	0	4.2	
	3	-	100
3.0	0	4.2	
	3	1.0	76.19
4.0	0	4.2	
	3	2.8	33.33
5.0	0	4.2	
	3	3.4	19.04
6.0	0	4.2	
	3	4.0	4.06
6.8	0	4.2	
(control)	3	4.2	-

Table 3. Survival of Encapsulated *L. acidophilus* at different pH with1% bile salts Concentration(x 10^8 cfu /ml)

pH	Incubation Period (hrs)	Population	% Reduction
2.0	0	4.2	
	3	0.3	92.85
3.0	0	4.2	
	3	1.5	64.28
4.0	0	4.2	
	3	3.6	14.28
5.0	0	4.2	
	3	3.8	9.52
6.0	0	4.2	
	3	4.04	3.80
6.8	0	4.2	
(control)	3	4.2	-

Table 4. Survival of Encapsulated *L.acidophilus* at different pH with 2% bile salts Concentration(x 10^8 cfu/ml)

pH	Incubation Period (hrs)	Population	% Reduction
2.0	0	4.2	
	3	0.09	97.85
3.0	0	4.2	
	3	1.4	66.66
4.0	0	4.2	
	3	3.5	16.66
5.0	0	4.2	
	3	3.7	11.90
6.0	0	4.2	
	3	4.02	4.28
6.8	0	4.2	
(control)	3	4.2	-

Table 5. Effect of encapsulation on the survival of *L.acidophilus* (x10^8 cells) at 1% bile salts Concentration

pH	Bile salts concentration	Free cells	Encapsulated cells	% increase in survival
3.0	1%	1.2	1.5	7.14
6.8 (control)	1%	4.2		

Table 6. Effect of encapsulation on the survival of *L.acidophilus* (x10^8 cells) at 2% bile Salt Concentration

Ph	Bile salts concentration	Free cells	Encapsulated cells	% increase in survival
3.0	2%	1.0	1.4	9.52
6.8 (control)	2%	4.2		

PLATES

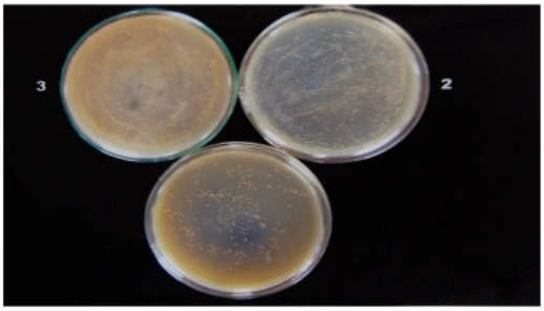
1. Number of colonies at pH 2.
2. Number of colonies at pH 3.
3. Number of colonies at pH 6.8.

1.Survival of free L.acidophilus under high acid and bile (1%) conditions.

Figure 1.

2.Survival of encapsulated L.acidophilus under high acid and bile (1%) conditions.

Figure 2.



3.Survival of encapsulated L.acidophilus under high acid and bile (2%) conditions.

Figure 3.

Reason may be that encapsulation protects the organisms from High acid, high salt, high bile and other chemical as well as physical conditions. Due to protective nature of capsule, encapsulation of microorganism by various systems (such as Alginate-Starch system) provides long term survival in stimulated gastro intestinal conditions. The above results have shown that alginate encapsulation enhanced the survival of L.*acidophilus* at pH 3 by 7.14 % (1% bile salts concentration) and by 9.52 % (2% bile salts concentration) (Table 5, 6).

Conclusion

The consumption of probiotics is vigorously growing due to the enormous benefits conferred on the health of consumers. With reference to this context, Microencapsulation is a process that favours the viability of probiotics in food products, mainly by protecting them against impaired environmental conditions like high pH and Salt concentrations. The use of sodium alginate polymers is one of the largest potential application in the encapsulation of probiotics because of their extreme versatile nature. Hence the aim of the present study was to evaluate viable encapsulation of probiotics with alginate and to investigate the effect of encapsulation on the survival of *L. acidophilus* under stimulated gastric conditions. Microencapsulation of *L.acidophilus* in sodium alginate resulted in better survival of cells at high pH and Salt concentrations as compared to free cells.

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