Xanthan chitosan polyionic hydrogels for microencapsulation of probiotics

Soma P. K. [1#] and Lo Y. M.[2*]
[1] 0112 Skinner building [2] University of Maryland – College Park, USA
* supervisor # spk9784@umd.edu

INTRODUCTION

Gastrointestinal tract is a host for numerous bacteria that work along with the digestive system in delivering nutrients and chemicals that affect our body in a positive way (Dunne C., 2001). Often, there is a competition between these good bacteria and the bad ones that are harmful. For many reasons like change in eating habits, consumption of pharmaceutical compounds, especially antibiotics, this balance is disturbed and may lead to colonization of disease causing bacteria (Fooks L. J., 2002). Hence good bacteria need to be consumed that can competitively inhibit harmful bacteria to re-establish this balance in the intestine (Fooks L. J., 1999). Dietary supplements of live bacteria that could re-establish this balance are termed as Probiotics. Probiotics are proven to provide a lot of health benefits like improving immune system, reducing lactose intolerance and providing anti-pathogenic effects (Scheinbach S., 1998). However, ingestion of these bacteria would likely be of no consequence, since most probiotics will be lysed when encountering the highly acidic gastric juices in the stomach, followed by the high bile salt concentration upon entering the small intestine.

Microencapsulation is considered one of the effective methods that can provide protection against these harsh gastrointestinal conditions. Over the years, many researchers have developed microencapsulation techniques to protect bacteria. These differ in the kind of coating material used or technique of encapsulation. Used mainly for microencapsulation of flavors and other sensitive chemicals in the food industry, polysaccharides such as starch and modified starches, alginate, chitosan have been applied to microencapsulate probiotic bacteria by various researchers (Mortazavian A., 2007; Hansen L. T., 2002; Crittendon R., 2001; Lee J. S., 2004). Although most of these encapsulation techniques have shown to improve viability of probiotic bacteria, they suffer from limitations like susceptibility to ions, low mechanical strength, and inability to release specifically in the intestine resulting in poor final effects. There are also problems with the stability and shelf life of products containing probiotics, consequently limiting their applicability in different types of foods. Scale-up production of such products is also highly difficult (Kailasapathy K., 2002; Roy D., 1987). Therefore, there is a pressing need for a better microencapsulation system to protect and deliver bacteria in intestine.

Xanthan and chitosan being natural biopolymers are biocompatible and non-toxic and are good candidates for microencapsulation of probiotics. Xanthan, a negatively charged polysaccharide, forms instant hydrogels when its aqueous solution comes in contact with that of chitosan, a positively charged polysaccharide. Xanthan-Chitosan hydrogels have been used previously for immobilization of xylanase enzyme (Dumitriu S., 1994) and *Corynebacterium glutamicum* (Chu C. H., 1996). In this study, we show that xanthan-chitosan hydrogels have protective effects on probiotic bacteria. We compared single and double layer encapsulation using xanthan chitosan hydrogels for protective effects on probiotic bacteria in simulated gastrointestinal conditions.

MATERIALS AND METHODS

Chitosan from crab shells with 85% deacetylation (batch # 086K0067), low molecular weight and medium molecular weight chitosan were purchased from Sigma-Aldrich Chemicals (St Louis, MO). Xanthan Gum (Lot # 55H1242) was purchased from Sigma Chemical Co. (St. Louis, MO). Artificial Gastric Juice (AGJ) and Simulated Intestinal Fluid (SIF) were purchased from Fisher Scientific (Rochester, NY) and Ricca Chemicals (Arlington, Texas), respectively. *Lactobacillus acidophilus* ATCC 43121 is obtained from ATCC (Manassas, VA). Xanthan solution was prepared by solubilizing the predetermined amount of xanthan in deionized water by constant stirring. Chitosan solution was prepared by solubilizing chitosan in 1N hydrochloric acid by heating at 65-70^{0}C for 30 min and stirring. The pH of the clear solution was adjusted using 1N NaOH solution and Di water was added to bring the solution to the final volume.

Culture preparation. Active culture of *Lactobacillus acidophilus* ATCC 43121 strain was maintained throughout the course of the experiments. *Lactobacillus acidophilus* ATCC 43121 was transferred twice in MRS broth at 35^{o}C under anaerobic conditions (using anaerogen, Oxoid, Hampshire, England). Culture cells were harvested after 36 hours by centrifugation at 10000 RPM for 20 min at 4^{o}C using Beckman L7-65 ultracentrifuge, washed twice and re-suspended in deionized water to a final concentration of ~10^9 colony-forming units (cfu/ml).

Microencapsulation Procedure. Actively growing cell suspension was added to 1.2% xanthan solution to get the final concentration of 1 % xanthan solutions with live cells (10^9 cfu/ml). Twenty ml of xanthan solution was added to 100 ml of 0.7% chitosan solution by drop wise addition using manually operated syringe with a 0.7 mm cannula (Becton-Dickinson, Franklin Lakes, NJ). The capsules X/Ch formed were allowed to stay in chitosan solution for 40 min at room temperature with continuous stirring to allow cross-linking and to avoid coalescence. The capsules were filtered through a 160 µm Millipore nylon filter (Billerica, MA), washed using DI water.

X/Ch/X Double layer capsules. Washed single layer capsules X/Ch were suspended in 50 ml DI water prior to adding 10 ml of 1% xanthan solution under continuous agitation for 30 min. The capsules were filtered through a 100 µm Millipore nylon filter and washed using DI water.

Simulated Gastric and Simulated Intestinal fluid assay. For viability of free cell in simulated gastric and intestinal fluid, suspension obtained after harvesting from MRS broth at 10000 RPM was added to simulated gastric juice at pH 1.5 for 1.5 hours, centrifuged, and added to simulated intestinal fluid for 5 hours. Sample from intestinal fluid solution was used to attain the total cell count (CFU/ml) of the viable bacteria.
For viability of encapsulated cells, the filtered and washed capsules were suspended in 50 ml simulated gastric juice at pH 1.5 (pH adjusted using 1N NaOH) and incubated at 35^{o}C for 1.5 hours. The capsules were filtered, washed and transferred into simulated intestinal fluid solution and incubated at 35^{o}C for 5 hours. Sample from the solution was used to get the total plate count (CFU/ml) of the viable bacteria.

Morphology of microcapsules. Surface morphology of microcapsules were examined using scanning electron microscope (SEM). Microcapsules were mounted on metal stub using double sided tape and coated with gold and titanium mixture. Samples of fresh freeze dried capsules were tested.

RESULTS AND DISCUSSION

Xanthan and chitosan polymers being oppositely charged form hydrogels instantaneously when their aqueous solutions are mixed. In our experiments, 1% xanthan solution containing cells and 0.7 % chitosan solution at pH 4.7 formed uniform capsules. Absence of free cells in the chitosan solution after filtration indicated very high encapsulation efficiency. Single layer (X/Ch) and double layer (X/Ch/X) capsules were formed to test the effect of an extra layer of protection on probiotic bacteria *Lactobacillus acidophilus* ATCC 43121.

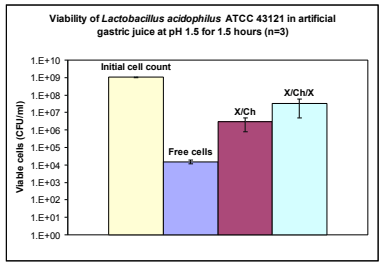

Figure 1 : Viability of Lactobacillus acidophilus ATCC 43121 in X/Ch and X/Ch/X capsules as compared to free cells in simulated gastrointestinal conditions

The protective effect of xanthan chitosan hydrogel capsules is shown in figure 1. Cells encapsulated in X/Ch capsules show at least 2 to 3 log increase in viability as compared to free cells. X/Ch/X capsules showed increased viability as compared to X/Ch capsules. SEM images of the surface of X/Ch and X/Ch/X capsules as shown in figure 1 indicates clear differences in surface characteristics with X/Ch/X being more smoothened compared to X/Ch indicating a thick layer of xanthan that could give added protection to cells during harsh conditions of stomach and intestine.

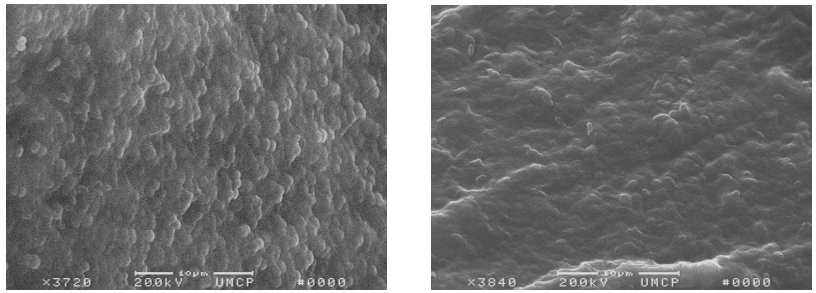

Figure 2: SEM images of surface of X/Ch (left) and X/Ch/X (right) capsules.

CONCLUSION

Xanthan chitosan hydrogels formed due to polyionic bonds have the ability to protect probiotic bacteria in gastrointestinal conditions. Addition of a layer of xanthan to form X/Ch/X has resulted in increased viability as compared to X/Ch capsules. Ease of formation of capsules and effective release of cells in bile salt solutions makes it a good encapsulation technique especially for targeted delivery in intestines.

REFERENCES

Chu, C. H et al. 1996. *Application of polyelectrolyte complex gel composed of xanthan and chitosan to the immobilization of Corynebacterium glutamicum.* Journal of Applied Polymer Science. 60, 1041-1047

Crittendon, R. et al. 2001. *Adhesion of Bifidobacteria to granular starch and its implications in probiotic technologies*. Applied and environmental Microbiology, 67(8), 3469-3475.

Dumitriu, S. et al. 1994. *Polyionic hydrogels obtained by complexation between xanthan and chitosan: Their properties as supports for enzyme immobilization*. Journal of Bioactive and Compatible Polymers. 9, 184-209.

Dunne, C. 2001. *Adaptation of bacteria to the intestinal niche: probiotics and gut disorder.* Inflammatory Bowel Diseases, 7, 136-145.

Fooks, L. J. et al. 1999. *Prebiotics, probiotics and human gut microbiology*. International Dairy Journal, 9, 53-61.

Fooks, L. J. et al. 2002. *Probiotics as modulators of the gut flora.* British Journal of Nutrition. 88, 1, S39-S49.

Hansen, L. T. et al. 2002. *Survival of ca-alginate microencapsulated Bifidobacterium spp. in milk and simulated gastrointestinal conditions*. Food Microbiology, 19, 35-45.

Kailasapathy, K. 2002. Microencapsulation of probiotic bacteria: technology and potential applications. *Current issues in intestinal microbiology.* 3, 39-48.

Lee, J. S. et al. 2004. *Survival of freeze-dried Lactobacillus bulgaricus KFRI 673 in chitosan coated calcium alginate microcapsules*. Journal of Agricultural and food chemistry. 52, 7300-7305.

Mortazavian, A. et al. 2007. *Principles and methods of microencapsulation of probiotic microorganisms*. Iranian Journal of Biotechnology. 5, 1, 1-18.

Roy, D. et al. 1987. *Continuous production of lactic acid from whey permeate by free and calcium alginate entrapped lactobacillus helveticus.* Journal of Dairy Science, 70, 506-513.

Scheinbach, S. 1998. *Probiotics: functionality and commercial status.* Biotechnology Advances, 16 (3), 581-608.