Mucoadhesive Alginate/Poly-L-Lysine/Thiolated Alginate Microcapsules for oral delivery of *Lactobacillus salivarius* 29

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Statement of Purpose: Lactic acid bacteria have been received tremendous scientific and commercial interest due to their enormous health benefits in the gastrointestinal tract. They are recognized as friendly bacteria which can inhibit and reduce numbers of potentially harmful bacteria from the intestine (1). *Lactobacillus salivarius* 29 (LS29) is a novel lactic acid bacterial strain which can be used as a probiotic microorganism for therapeutic applications. LS29 strain also produces unknown anti-microbial molecules which can inhibit several potentially harmful pathogenic bacteria such as *E. coli K88*, *E. coli O157*, *Salmonella enteritidis*, *Salmonella typhimurium* and some others. To exert positive health benefits, therapeutic live bacteria must be delivered to the intestine alive through oral administration. However, low oral bioavailability, poor survival and stability in acidic stomach and short residence time at the target site have made oral delivery of live bacteria as a most challenging and difficult field in the research arena. Considering these-mentioned problems and importance, mucoadhesive thiolated alginate (TA) was synthesized to prepare alginate/poly-L-lysine/thiolated alginate (APTA) microcapsules for efficient oral delivery of LS29.

Methods: TA was prepared by covalent attachment of cysteine to alginate by the formation of amide bonds between the carboxylic acid groups of the alginate and the primary amino groups of the cysteine. Briefly, the carboxylic acid groups of the alginate were activated by EDC/NHS chemistry and then cysteine was introduced to the reaction mixture to prepare cysteine conjugated alginate (Figure 1). The thiol content of the polymer conjugate was quantitatively measured by Ellman's reagent reaction. The modified polymer was also qualitatively characterized by FTIR. The survival of free LS29 in simulated gastric (SGF) and simulated intestinal fluid (SIF) was investigated to show the importance of microencapsulation of this novel strain.

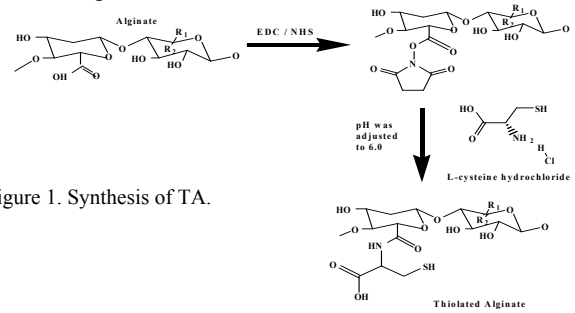

Figure 1. Synthesis of TA.

LS29-loaded APTA microcapsules were prepared by extruding the alginate-LS29 mixture into calcium chloride solution and subsequently the beads were immersed in poly-L-lysine and finally coated with TA as a surface membrane. APTA microcapsules were then characterized for their efficacy in oral administration of LS29.

Results: About 759 ± 32.4 µM cysteine was introduced to per gram of the alginate. FTIR analysis showed several new peaks in the modified polymer. LS29-loaded APTA microcapsules were spherical with smooth surface and the average microcapsule size was 1.12 ± 0.05 mm (Figure 2). APTA microcapsules provided higher loading content and efficiency (99.7 ± 0.36 %). About 40 % survival of LS29 in SGF (pH 2.0) encapsulated in APTA microcapsules was maintained up to 60 min (Figure 3), whereas no LS29 survived in SGF (pH 2.0) up to 20 min (Figure 4). Encapsulated LS29 was released from APTA microcapsules in SIF (pH 7.2) in a time dependent manner and maintained their survival (Figure 5). The immune stimulation in macrophage cell-line and the mucoadhesive properties of LS29-loaded APTA microcapsules will be reported.

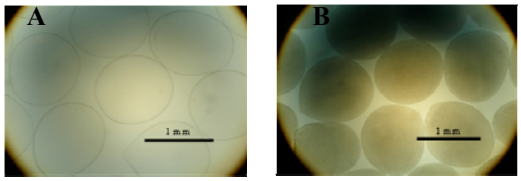

Figure 2. Empty (A) and LS29-loaded APTA microcapsules (B)

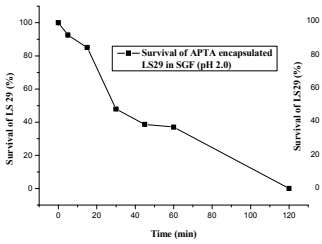

Figure 3. Survival of encapsulated LS29 in SGF.

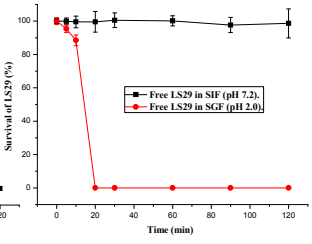

Figure 4. Survival of LS29 in SGF and SIF.

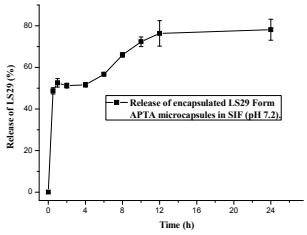

Figure 5. Release of encapsulated LS29 in SIF.

Conclusions: Cysteine was successfully conjugated to the alginate to prepare the mucoadhesive TA. LS29-loaded APTA microcapsules were successfully prepared and provided a very promising delivery system for oral administration of *Lactobacillus salivarius* 29.

Reference

1. Gillian Y. Nat Rev Microbiol. 2008: 6: 174-175.