# Abstract

**Over the past several years, there has been a concerted effort to develop a new vaccine against tuberculosis. The existing vaccine, Mycobacterium bovis Bacillus Calmette-Guerin (BCG), has been used for many decades, but meta-analysis of controlled clinical trials has revealed a lack of effectiveness in adults. In order to develop the oral vaccine that could be administrated orally, be stable during oral administration and be targeted to M cells, we prepared the poly-DL-(lactide-co-glycolide)?]PLGA?^ microspheres containing BCG by a water-in-oil-in-water emulsion solvent evaporation method. The BCG strain was inoculated in Middlebrook 7H9 broth. By polymerase chain reaction, we found that the BCG strain used in this study was derived from Japanese strain. By Western blotting, two proteins of 105 kDa and 45 kDa were found in the culture media. To further develop the M-cell targeting microspheres, we cloned, expressed, and purified the Escherichia coli heat-labile enterotoxin subunit B (LTB) from prokaryotic expression system. The purified recombinant LTB exhibited a similar binding kinetic as the wild-type LTB did, suggested that recombinant LTB was able to bind to the ganglioside on the epithelial cells. The BCG was then encapsulated by solvent evaporation method. The microspheres showed the spherical shape and exhibited monolithic form. In vitro release study of BCG-loaded microspheres displayed a controlled release kinetic with a burst effect during the first six days and a sustained release for the next 12 days. These results suggested that the PLGA-based microspheres could serve as a formulation for controlled-release vaccine.**

**Key Word：Bacillus Calmette-Guerin、oral vaccine、Escherichia coli heat-labile enterotoxin、microsphere**