Thesis Title	The Quantitative PCR Determination for Probiotic Promotion of Beta-glucan from *Cordyceps militaris* and Analysis on Antioxidant Property
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ABSTRACT

Probiotics are regarded for human health advantages; however, these benefits rely on the specific bacterial strains. *Streptococcus thermophilus* has been extensively used as a starting culture in the dairy products. However, bacterial growth and idenfication of probiotic is commonly used culture technique with labourious and time-consuming. The objective of this study was (1) to develop qPCR technique to determine the *S. thermophilus* growth and (2) evaluate growth promoting using the beta-glucan extract from fungal mycelia of *Cordyceps militaris* by using qPCR technique.

In this study, the genome-based method has been developed to use a specific gene of *S. thermophilus*, the Glucose kinase gene (*GlcK*), by using quantitative PCR (qPCR). The 139-bp PCR product was successfully cloned and used to generate a DNA standard curve by plotting the threshold cycle (Cq) versus log DNA concentration of plasmid DNA, with an amplification efficiency of 97.2%. In addition, coefficient of variation was calculated considering both threshold cycle (Ct) and bacterial cell enumerated by plate counts, which indicated the log CFU/mL (1.69–6.56) and log DNA copies (2.07–6.03). This linear relationship revealed a quantitative curve (R^2 =0.989) with a detection in range from 1.69 to 6.56 log CFU/mL. Next, to determine the prebiotic index and activity, here, the beta-galactosidase, *LacZ* gene of *E. coli* was cloned using 122-bp PCR product and generated standard curved from Ct and log DNA concentration which revealed indicated the log CFU/mL (2.35–7.35) and log DNA copies (2.61–7.08). This linear relationship revealed a quantitative curve (R^2 =0.9912) with a detection range from 2.35 to 7.35 log CFU/mL. Subsequently, correlations

between bacterial growth obtained from qPCR and plate count method were conducted. Hence, qPCR-based methods facilitated reliable quantification *S. thermophilus* and *E. coli* in growth determination during culture.

Next, we extracted the beta-glucan from *C. militaris*, achieving a yield of about 2.5% and demonstrating an antioxidant activity of 85%. The prebiotic testing was conducted by supplementing with beta-glucan, lactose and inulin in culture media of *S. thermophilus* and *E. coli* (as a negative control) for 48 hrs. The qPCR method was used to monitor the culture every 4 hrs. We calculated the growth and prebiotic properties based on the copy numbers. The results revealed that beta-glucan had potential as a prebiotic, exhibiting a prebiotic index at 3.21, which is comparable to inulin at 0.37. Here the use of qPCR is an alternative method to determine the number of cells and cell growth during culture in the two bacterial strains.

Keywords: Beta-glucan, Glucose Kinase Gene, Probiotic, Quantitative PCR, *Streptococcus thermophilus*