Optimization of temperature and pH of the transconjugant produced by genus *Lactobacillus* and genus *Lactococcus*

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Abstract

Probiotics are live microorganisms known to improve gut health which has become more evident in recent times. The present study aimed to optimize a trans-conjugant made via conjugation of two probiotic strains, genus *Lactobacillus* and genus *Lactococcus*. As *Lactobacillus* is a probiotic strain that shows slow growth in milk due to its inability to metabolize lactose and casein, the novel trans-conjugant was expected to possess improved growth rates by conjugating recipient bacterium *Lactobacillus* and donor bacterium *Lactococcus* for faster growth of *Lactobacillus* in milk for probiotic production. *Lactobacillus* and *Lactococcus* strains were isolated from curd and cheese respectively, confirmed with biochemical tests including Gram staining, catalase test, sugar fermentation test, Arginine hydrolysis tests and Arginine dihydrolase test, sub-cultured and then conjugated. Growth curves of the trans-conjugant at varied temperatures and pH were generated using spectrophotometry to determine optimum conditions for the growth of trans-conjugant. The results confirmed the presence and growth of trans-conjugant through selective growth in modified De Man Rogosa and Sharpe Agar (MRS) which indicated a color change from purple to yellow. Analysis of growth of *Lactobacillus* and trans-conjugant in MRS indicated a shorter lag phase in trans-conjugant by 2h when compared to that of *Lactobacillus*. Spectrophotometric analysis of trans-conjugant at varying temperatures indicated a significant difference ($p = 0.0389$) in growth rate at 30ºC when compared to 4ºC and 37ºC whereas trans-conjugant at varying pH indicated the highest yield and best growth rate reaching stationary phases within 28h at pH 6 when compared to pH 4 and 5 with a log phase longer than 54h and pH 7 which reached stationary phase within 2h. The growth condition optimization of trans-conjugant with a significantly ($p = 0.0304$) higher growth rate by 2h compared to *Lactobacillus* revealed an optimum pH 6 and 30°C for faster microbial biomass production needed for probiotics.

Keywords: *Lactobacillus, Lactococcus, Probiotics, Spectrophotometry, Trans-conjugant*