ABSTRACT

Since its discovery 35 years ago, the functional significance of catalytic RNA molecules, called ribozymes, has been better manifested through the analysis of their crystal structures. Hammerhead ribozyme of a plant satellite viroid catalyses a site-specific self-cleavage reaction, driven by an acid-base catalysis, producing RNA products with 2'3'-cyclic phosphate group and 5'-hydroxyl group. In this study, hammerhead ribozyme is utilised to validate the action of RNase J1, a ribonuclease that degrades the 5'-hydroxyl transcripts involved in *glmS* gene regulation during cell wall biosynthesis in *Bacillus subtilis.* An *in vivo* Hammerhead ribozyme system and an inducible-RNase J1 expression is established in BL21(DE3) cells using a two-stage Lambda Red recombineering. The first stage permitted the integration of Hammerhead ribozyme fused with reporter EGFP-hDHFR gene at *arsB* locus of BL21(DE3) genome while the second stage integrated RNase J1 gene behind an Arabinose-inducible P_{ARA} promoter at *lacZ* locus. Fluorescence assay was performed to evaluate the fluorescence (EGFP-hDHFR) production in the established strains upon graded expression of RNAse J1 under different arabinose concentrations. 49% and 60% reduction of fluorescence intensity were apparent in strain with active Hammerhead ribozyme treated with 0.0012% (w/v) and 0.0037% (w/v) arabinose, respectively, implying RNase J1 degradation of the 5'-hydroxyl tagged EGFP-hDHFR mRNA produced through the ribozyme cleavage. The implication of RNase J1 activity in *E. coli* in this study upholds the concept of constructing a cell based screening assay for the identification of compounds that target the *glmS* ribozyme of *B. subtilis.*