

The expression of recombinant proteins from Escherichia coli along with the possibility of their subsequent secretion into the extracellular space is a highly desirable feature due to reasons like easier downstream purification and protection of the product from degradation by the host. This feature has been studied and characterized previously in this group the expression of Bacteriocin Release Protein (BRP) controlled by a growth-phase regulated promoter ($P_{\text{raisebox}\{-0.3ex\}\text{scriptsize fic}}$) that aids in the release of recombinant proteins from the periplasmic space through the outer membrane. It was of major interest to know whether BRP-mediated secretion of recombinant proteins could be adopted in a stable chemostat and if so, what kind of interplay would be seen between activity of growth-phase regulated promoters and the productivity of a chemostat process. To make this continuous process even more sustainable, alternative plasmid selection mechanisms were tested with the aim of avoiding antibiotic-based plasmid selection. Through the use of auxotrophy complementation, it was possible to maintain a previously unstable plasmid, for extended periods of continuous cultivation without any antibiotic-based selection pressure and show extracellular expression of a recombinant enzyme, thus providing an interesting new model.

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