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Development of a novel fusion system for antibiotic-free *Escherichia coli* recombinant protein production

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Escherichia coli is one of the most widely host systems used for production of recombinant proteins. Antibiotics are commonly used during bacterial fermentation, and the vast majority of expression vectors, particularly for *E. coli*, contain antibiotic resistance genes as selection markers. Despite being a powerful selection tool, their use has been considered unacceptable in many areas of biotechnology, as the recombinant protein production for therapeutic use and vaccines, by regulatory authorities. In fact,this selection system has several disadvantages, namely,the risks for human health due to thespread ofthe resistance genes,loss of selective pressure as a result of antibiotic degradation and the excessive metabolic burden exerted by the constitutive expression of the antibiotic resistance gene on the host cell. Those drawbacks can be stopped by using antibiotic-free expression systems, which represent lower production costs and, in many cases, permit to obtain highly desirable characteristics, such as the sharp increase in recombinant protein production.The novel fusion system presented in this study is composed by one low molecular weight moiety, the Fh8 fusion partner. Previous studies have demonstrated the ability of this fusion partner to improve soluble protein expression using antibiotic selection systems. Taking into account the advantages of producing recombinant proteins without antibiotics, the novel fusion partner was applied to an antibiotic-free system from Delphi Genetics in order to evaluate its contribute, as a solubility *tag*, on recombinant protein production. Using DNA recombinant technology, the sequence of the Fh8 partnerwas inserted into pStaby plasmids and the codifying genes for three target proteinswere genetically fused to the novel partner. The bio-productions were carried out on cultures of 100 mL and the resulting fusion proteins were analysed by SDS-PAGE and compared to the non-fused protein controls. Fusion proteins used in this work were purified using nickel affinity resins and quantified by Bradford. All fusion proteins presented higher soluble expression than the non-fused ones. Results from this work showedthat the Fh8 fusion system can be used in an antibiotic-free expression system, offeringan effective and safe methodology to produce soluble recombinant proteins in *E. coli*.