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High Level Expression of Fluorinase in Escherichia Coli and Pichia Pastoris

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Abstract : The first fluorinating enzyme, 5'-fluoro-5'-deoxyadenosine synthase (fluorinase) was isolated from the soil bacterium Streptomyces cattleya. Such an enzyme, with the ability to catalyze a C-F bond, presents great potential as a biocatalyst. Naturally fluorinated compounds are extremely rare in nature. As a result, the number of fluorinases identified remains relatively few. The field of fluorination is almost completely synthetic. However, with the increasing demand for fluorinated organic compounds of commercial value in the agrochemical, pharmaceutical and materials industries, it has become necessary to utilize biologically based methods such as biocatalysts. A key step in this crucial process is the large-scale production of the fluorinase enzyme in considerable quantities for industrial applications. Thus, this study aimed to optimize expression of the fluorinase enzyme in both prokaryotic and eukaryotic expression systems in order to obtain high protein yields. The fluorinase gene was cloned into the pET 41b(+) and pPinkα-HC vectors and used to transform the expression hosts, E.coli BL21(DE3) and Pichia pastoris (PichiaPink™ strains) respectively. Expression trials were conducted to select optimal conditions for expression in both expression systems. Fluorinase catalyses a reaction between S-adenosyl-L-Methionine (SAM) and fluoride ion to produce 5'-fluorodeoxyadenosine (5'FDA) and L-Methionine. The activity of the enzyme was determined using HPLC by measuring the product of the reaction 5'FDA. A gradient mobile phase of 95:5 v/v 50mM potassium phosphate buffer to a final mobile phase containing 80:20 v/v 50mM potassium phosphate buffer and acetonitrile were used. This resulted in the complete separation of SAM and 5’-FDA which eluted at 1.3 minutes and 3.4 minutes respectively. This proved that the fluorinase enzyme was active. Optimising expression of the fluorinase enzyme was successful in both E.coli and PichiaPink™ where high expression levels in both expression systems were achieved. Protein production will be scaled up in PichiaPink™ using fermentation to achieve large-scale protein production. High level expression of protein is essential in biocatalysis for the availability of enzymes for industrial applications.

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