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SHORT COMMUNICATION

Enhanced activity of *meso*-secondary alcohol dehydrogenase from *Klebsiella* species by codon optimization

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Abstract *Meso*-secondary alcohol dehydrogenases (*meso*-SADH) from *Klebsiella oxytoca* KCTC1686 and *Klebsiella pneumoniae* KCTC2242 were codon optimized and expressed in *Escherichia coli* W3110. The published gene data of *K. pneumoniae* NTUH-K2044 (NCBI accession number AP006725), *K. pneumoniae* 342 (NCBI accession number CP000964), and *K. pneumoniae* MGH 78578 (NCBI accession number CP000647), were compared with the *meso*-SADH sequences of each strain, respectively. Codon-optimized *meso*-SADH enzymes of *K. oxytoca* and *K. pneumoniae* showed approximately twofold to fivefold increased enzyme activities for acetoin reduction over native enzymes. The highest activities for each strain were obtained at 30–37 °C and pH 6–7 (yielding 203.1 U/mg of protein and 156.5 U/mg of protein, respectively). The increased enzyme activity of the codon-optimized enzymes indicated that these modified enzymes could convert acetoin into 2,3-butanediol with a high yield.

Keywords Codon optimization · *Meso*-secondary alcohol dehydrogenase · 2,3-Butanediol · Gene expression · *Escherichia coli*

Introduction

2,3-Butanediol is a valuable C_4 compound essential for the production of platform chemicals [1, 2]. The production of 2,3-butanediol (2,3-BD) from microorganisms shows promise for industrial applications and has the potential to alleviate dependence on oil supplies for use in generating platform chemicals [1, 2]. 2,3-BD (also known as 2,3-butylene glycol, dimethylene glycol, or dimethylethylene glycol) has many applications in various fields—including the production of synthetic rubber, plastics, and solvents—and is useful as an antifreeze agent, a precursor to 1,3-butadiene, and a highly valued flavoring agent in food products [3]. Furthermore, dehydration of 2,3-BD produces methyl ethyl ketone (MEK; butan-2-one), an effective fuel additive with a higher heat of combustion than ethanol [4]. Also, due to its high octane number, 2,3-BD can be used as an 'octane booster' for petrol.

Escherichia coli, which was used as the host in this study, is the most commonly used expression system for the production of heterologous proteins. In order to increase the product production in *E. coli*, various strategies that provide high protein yields have been analyzed and reported [5, 6]. For the optimization of transcriptional or translational progress, one strategy is to use codon optimization, a technique that maximizes the usage of codons that *E. coli* prefers [5]. Codon optimization is an important factor that affects gene expression levels, which ultimately affect protein production, and this experimental process has been described in several recent papers.

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