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Construction and expression of a polycistronic plasmid encoding *N*-acetylglucosamine 2-epimerase and *N*-acetylneuraminic acid lyase simultaneously for production of *N*-acetylneuraminic acid

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HIGHLIGHTS

- Encoding enzymes for two reactions in one plasmid simplified fermentation process.
- Activities of two types of *N*-acetylglucosamine 2-epimerase were compared.
- Intact *E. coli* cells avoid adding ATP to activate *N*-acetylglucosamine 2-epimerase.
- Lowering *N*-acetylglucosamine 2-epimerase expression level improved its solubility.
- High production of 61.3 g/l *N*-acetylneuraminic acid.

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ABSTRACT

Synthesis of *N*-acetylneuraminic acid (Neu5Ac) from *N*-acetylglucosamine (GlcNAc) and pyruvate was carried out by constructing and expressing a polycistronic plasmid encoding an *N*-acetylglucosamine 2-epimerase (AGE) gene and an *N*-acetylneuraminic acid lyase (Nal) gene simultaneously. Nal from *Escherichia coli* K12 and AGEs from *Synechocystis* sp. PCC 6803 (snAGE) and *Anabaena* sp. CH1 (anAGE) were used. And four polycistronic plasmids were constructed in which the positions of AGE gene differed with respect to Nal gene. Among these plasmids, pET-28a-Nal-anAGE with anAGE gene located next to Nal gene caused the production of the highest amount of Neu5Ac, generating 61.3 g/L in 60 h by whole-cell catalysis without the addition of ATP as AGE activator. And pET-28a-Nal-anAGE lowered anAGE's expression level, allowing it to fold properly. Thus, an inclusion-body-free *E. coli* strain capable of producing Neu5Ac by whole-cell catalysis with high yield and low cost was constructed in the present study.

1. Introduction

Mammalian cells are covered with sugar chains which are terminated by a family of 9-carbon amino sugars called sialic acids. These sugar derivatives are often part of the recognition sites where pathogens attach (Varki and Varki, 2007). Sialic acids are involved in the modulation of various biological processes, such as virus invasion, cell differentiation, fertilization, cell adhesion, inflammation, and tumorigenesis (Hu et al., 2010). More than 40 types of sialic acids have been discovered in nature, and Neu5Ac is the most ubiquitous and the biosynthetic precursor for all other sialic acids (Maru et al., 2002; Ogura, 2011). Neu5Ac is a potential raw material in the synthesis of zanamivir, which prevents both influenza type A and B infections (Tao et al., 2010). Neu5Ac is also an important additive in dairy products, as it is able to strengthen the immunity of infants (Oriquat et al., 2011). In addition, Neu5Ac is of great diagnostic value as an important indicator for many diseases (Gopaul and Crook, 2006).

Traditionally, Neu5Ac has been prepared by extraction from natural sources (such as milk or eggs), or by hydrolyzing capsular

Abbreviations: Nal, *N*-acetylneuraminic acid lyase; AGE, *N*-acetylglucosamine 2-epimerase; snAGE, *N*-acetylglucosamine 2-epimerase from *Synechocystis* sp. PCC 6803; anAGE, *N*-acetylglucosamine 2-epimerase from *Anabaena* sp. CH1; GlcNAc, *N*-acetylglucosamine; ManNAc, *N*-acetyl-D-mannosamine; Neu5Ac, *N*-acetylneuraminic acid.

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