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Deletion of the *aroK* gene is essential for high shikimic acid accumulation through the shikimate pathway in *E. coli*

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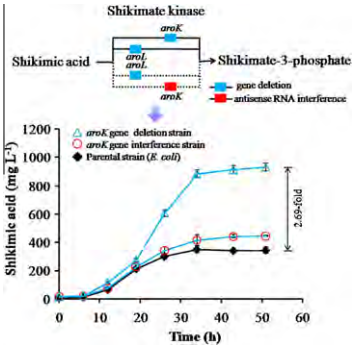
HIGHLIGHTS

- Antisense RNA interference was first employed to inactivate *aroK* gene.
- The *aroK* gene deletion achieved greater shikimic acid accumulation.
- The shikimate kinase activity in the *aroK* gene deletion strain was lower.
- Glycerol is an effective carbon source for shikimic acid accumulation.

GRAPHICAL ABSTRACT



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ABSTRACT

Shikimic acid (SA) is an important metabolic intermediate with diverse commercial applications. In this work, antisense RNA interference and gene deletion were carried out to inactivate the *aroK* gene in an SA-producing *Escherichia coli* strain, DHPYA-T7. In this strain, the *aroL*, *ptsHIcrr* and *ydiB* genes are deleted, and the *tktA*, *glk*, *aroE* and *aroB* genes are overexpressed. Flask cultivations of the DHPYA-T7 derivative strains showed that the accumulation of SA increased 2.69-fold after *aroK* gene deletion (DHPYAAS-T7) and 1.29-fold after antisense RNA interference (DHPYAS-T7). Furthermore, the activity of shikimate kinase in DHPYAAS-T7 was 0.21-fold of that in strain DHPYAS-T7. In a 10-L fermentation, SA accumulation increased to 1850 mg L^{-1} in strain DHPYAAS-T7, which is a 1.5-fold increase over that in strain DHPYAS-T7. These results demonstrate that *aroK* gene inactivation in DHPYA-T7 leads to high SA accumulation, especially when this inactivation is caused by chromosomal deletion.

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1. Introduction

Shikimic acid (SA) is an important intermediate in aromatic amino acid biosynthesis (Krämer et al., 2003; Chandran et al., 2003; Adachi et al., 2006). Moreover, SA is a key precursor for the chemical synthesis of the neuraminidase inhibitor, oseltamivir phosphate, known as Tamiflu® (De Clercq, 2002), which is now clinically employed for the treatment of the avian virus, type H5N1, and A/H1N1 influenza infections (Russell et al., 2006). Furthermore, SA can be used for the synthesis of renewable phenol (Gibson et al., 2001), and its derivatives can be used as herbicides and antibacterial agents without negative effects in mammals (Jiang et al., 1999; Song et al., 2001). However, the current preparation of SA is still, to a large extent, dependent on a multistep, low-yielding extraction process of the plant *Illicium* (Haslam, 1993; Krämer et al., 2003; Escalante et al., 2010). Thus, many researchers have designed and constructed *Escherichia coli* strains in which the accumulation of SA can be facilitated by preventing further downstream conversion into aromatic amino acids (Draths et al., 1999; Johansson et al., 2005; Knop et al., 2001; Yi et al., 2003,

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