Production of alginate oligosaccharides by immobilized recombinant alginate lyase

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李融昀(5109)

Outline

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二、The fusion of the *phaC* gene from *Ralstonia eutropha* H16 with the ALG gene

三、Expression and purification of PHA-ALG

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Abstract

Alginate oligosaccharides (AOS) possess excellent biological activity and water solubility, making them widely applicable in industries such as food, pharmaceuticals, and materials. Alginate lyase (Aly or Alg) degrades alginate, producing unsaturated alginate oligosaccharides (UAOS). Microbial cells capable of simultaneously expressing three enzymes: 3-ketothiolase (PhaA), acetoacetyl-CoA reductase (PhaB), and PHA synthase (PhaC), can form intracellular polyhydroxyalkanoate (PHA) nanoparticles. PHA nanoparticles are insoluble inclusion bodies that can be separated by methods like cell disruption and centrifugation. Enzyme or cell immobilization enables repeated use, enhances operational stability, and facilitates the separation of immobilized enzymes, cells, and products. This study aims to produce functional PHA nanoparticles with immobilized *Cellulophaga lytica* alginate lyase, termed PHA-ALG. The ALG gene was fused with the *phaC* gene to construct the pET-21b-*phaC-alg* plasmid. The pET-21b-*phaC-alg* plasmid and pBBR1MCS-*phaAB* were co-transformed into *Escherichia coli* host cells for expression. After producing PHA-ALG, the optimal host for expression and the optimal IPTG induction concentration will be investigated. Subsequently, the enzyme characteristics will be further explored, and PHA-ALG will be used to produce alginate oligosaccharides to examine product composition, conversion rate, and reusability.