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High-level exogenous glutamic acid-independent production of poly-(γ-glutamic acid) with organic acid addition in a new isolated *Bacillus subtilis* C10

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ABSTRACT

A new exogenous glutamic acid-independent γ-PGA producing strain was isolated and characterized as *Bacillus subtilis* C10. The factors influencing the endogenous glutamic acid supply and the biosynthesis of γ-PGA in this strain were investigated. The results indicated that citric acid and oxalic acid showed the significant capability to support the overproduction of γ-PGA. This stimulated increase of γ-PGA biosynthesis by citric acid or oxalic acid was further proved in the 10 L fermentor. To understand the possible mechanism contributing to the improved γ-PGA production, the activities of four key intracellular enzymes were measured, and the possible carbon fluxes were proposed. The result indicated that the enhanced level of pyruvate dehydrogenase (PDH) activity caused by oxalic acid was important for glutamic acid synthesized *de novo* from glucose. Moreover, isocitrate dehydrogenase (ICDH) and glutamate dehydrogenase (GDH) were the positive regulators of glutamic acid biosynthesis, while 2-oxoglutarate dehydrogenase complex (ODHC) was the negative one.

1. Introduction

Poly-γ-glutamic acid (γ-PGA) is an anionic biomaterial formed via the gamma-amide linked polymerization of glutamic acids. As a new macromolecular material, γ-PGA is completely biodegradable, water-soluble and nontoxic to humans, and possesses enormous potential applications in the industrial (Shih and Van, 2001; Buescher and Margaritis, 2007), such as a thickener and a stabilizer in the food industry (Sung et al., 2005), as a bioflocculants in waste-water treatment (Shih et al., 2001), as a drug carrier and controlled release materials in medicine (Manocha and Margaritis, 2008), as a fertilizer in agriculture (Chen et al., 2005), as a humectants in cosmetics (Konno et al., 1989), etc.

As the main producers of γ-PGA, various *Bacilli* strains could be classified into two groups based on the origin of glutamic acid. The exogenous glutamic acid-dependent strain must ingest glutamic acid from the outside environment for enough supply of substrate; while the exogenous glutamic acid-independent strain could self-synthesize such essential precursor. Presently, most studies about the production of γ-PGA were focused on the exogenous glutamic acid-dependent strains (Goto and Kunioka, 1992; Kubota et al., 1993; Cromwick et al., 1996; Jung et al., 2005; Shi et al., 2007; Wu et al., 2008; Su et al., 2010). Extensive researches and many efforts have been made to clarify the biosynthetic pathway and regulation mechanism of γ-PGA production, for the productivity improvement of γ-PGA (Kunioka, 1997; Ashiuchi et al., 2004; Ashiuchi and Misono, 2002; Candela et al., 2005; Du et al., 2005; Huang et al., 2011a). However, the high production cost of γ-PGA is still the major obstacle to limit the large-scale manufacture and utilization of this biopolymer (Huang et al., 2011b). Compared with production through the addition of exogenous glutamic acid, the biosynthesis of γ-PGA via the direct utilization of glucose is preferred by screening a glutamic acid-independent strain. Such ideal strategy to produce γ-PGA from a common and cheap raw material should significantly reduce the cost of γ-PGA and boost the application of this useful biopolymer. Moreover, the study of *de novo* γ-PGA production in the glutamic acid-independent strain would integrate the metabolic pathway of glutamic acid with the biosynthesis of γ-PGA, which shall reveal new insights into the biosynthetic and regulation mechanisms of this biopolymer.

So far, only a few γ-PGA producing *Bacillus* strains have been identified as exogenous glutamic acid-independent type and the metabolic mechanism for γ-PGA biosynthesis needs to be explored. These strains include *Bacillus licheniformis* A35 (Cheng et al., 1989), *Bacillus subtilis* TAM-4 (Ito et al., 1996), *Bacillus* sp. SAB-26 (Soliman et al., 2005) and *Bacillus amyloliquefaciens* LL3 (Cao et al., 2011). Although culture conditions have been respectively optimized in

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