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Enhanced α-ketoglutaric acid production in *Yarrowia lipolytica* WSH-Z06 by an improved integrated fed-batch strategy

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ABSTRACT

This study aimed at enhancing α-ketoglutaric acid (α-KG) production by *Yarrowia lipolytica* WSH-Z06. Batch culture experiments demonstrated that $CaCO_3$ and a relatively low pH (3.0) in the α-KG production phase contributed to α-KG synthesis. Using a two-stage pH control strategy, in which pH was buffered by $CaCO_3$ in the growth phase and then maintained at 3.0 in the α-KG production phase, the yield of α-KG reached 53.4 g L^{-1}. In the later phase of batch fermentation, the glycerol was exhausted but synthesis of α-KG did not cease. Therefore, glycerol was fed with an integrated fed-batch mode, and α-KG production increased to 66.2 g L^{-1} with a productivity of 0.35 g L^{-1} h^{-1}. Compared to optimal batch culture, α-KG production and productivity were enhanced by 23.9% and 16.7%, respectively. The two-stage pH control strategy, constant feeding approach and lower pH in later phase would be useful for α-KG industrial production.

1. Introduction

α-Ketoglutaric acid (α-KG), is an important multifunctional organic acid generated in the tricarboxylic acid (TCA) cycle. It has a broad range of industrial applications in the food, pharmaceutical, fine chemistry and animal feed industries (Finogenova et al., 2005; Otto et al., 2011). As a precursor of glutamic acid, α-KG has a sparing effect on endogenous glutamine pools, and is closely linked with the synthesis of proline, arginine and polyamines (Matzi et al., 2007). Additionally, α-KG plays a pivotal role in the intermediary metabolism to modulate nitrogen balance (De Bandt et al., 1998). In clinical nutrition and healthcare, α-KG can improve gut morphology and function, counteract trauma-induced dysimmunity and play an important role in metabolic adaptation to injury (Cynober, 1999). The L-arginine and α-KG mixture is a popular nutrition enhancer in functional beverages to improve blood flow to muscle, reduce catabolism and increase protein synthesis during resistance training, resulting in improved training adaptations (Campbell et al., 2006). Barrett and Yousaf (2008) proposed that α-KG could be further exploited as a poly-(triol-α-ketoglutarate) with new potential applications, e.g. tissue engineering and drug delivery.

Currently, α-KG is produced via multi-step chemical synthesis. However, it is hard to overcome shortcomings that include low yield, low purity, residual cyanides and other toxic waste (Otto et al., 2011). Biotechnological processes, as attractive alternative methods of α-KG production, have been studied for several decades (Finogenova et al., 2005). Many bacteria and yeasts have been screened for α-KG overproduction. Among them, *Yarrowia lipolytica* has been the most intensively studied non-conventional yeast due to its several advantages: e.g. wide substrate spectrum, intense secretory ability, higher product yield, waste minimization and an existing efficient system for genetic engineering transformation (Holz et al., 2010; Madzak et al., 2004; Mirbagheri et al., 2011). Moreover, *Y. lipolytica* is considered by the Food and Drug Administration (USA) as nonpathogenic and many processes based on this organism are classified as Generally Recognized as Safe (GRAS) (Barth and Gaillardin, 1997). In recent years, there have been many significant studies concerning the overproduction of α-KG (Otto et al., 2011). Chernyavskaya et al. (2000) identified the principal conditions affecting α-KG oversynthesis by the mutant *Y. lipolytica* N1 from ethanol, and a α-KG concentration of 49 g L^{-1} was achieved; however, the concentration of ethanol in the broth should be maintained at <2.5 g L^{-1}. Liu et al. (2007) found that little α-KG was detected as a byproduct in the pyruvic acid fermentation broth of *Torulopsis glabrata*. However, redistributing the metabolic flux to α-KG by manipulating cofactor levels resulted in pyruvic acid concentration decreasing from 69 to 21.8 g L^{-1}, while α-KG concentration increasing to 43.7 g L^{-1}. Other studies

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