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Acetoin production enhanced by manipulating carbon flux in a newly isolated *Bacillus amyloliquefaciens*

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HIGHLIGHTS

- An acetoin high-producing *Bacillus amyloliquefaciens* FMME044 was newly isolated.
- The mechanism of exchange of 2,3-butanediol and acetoin is explained.
- A novel strategy of manipulating carbon flux to acetoin is proposed.
- 51.2 g/L acetoin with yield of 0.43 g/g and productivity of 1.42 g/L/h was achieved.

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ABSTRACT

A new strain, FMME044, exhibited a remarkable ability to synthesize acetoin and was identified as *Bacillus amyloliquefaciens*. The following characteristics of enzyme activity were found: 2,3-butanediol was reverse transformed to acetoin upon depletion of glucose; lower agitation speeds favored 2,3-butanediol accumulation; and higher agitation speeds favored reverse transformation of 2,3-butanediol to acetoin. In order to enhance acetoin production by manipulating the carbon flux distribution, a two-stage agitation speed control strategy was proposed: during the first 24 h, the agitation speed was set to 350 rpm to achieve a high 2,3-butanediol concentration and then the speed was increased to 500 rpm to reverse transform 2,3-butanediol to acetoin. Following this strategy, a high titer (51.2 g L^{-1}), yield (0.43 g g^{-1}), and productivity (1.42 g L^{-1} h^{-1}) of acetoin were achieved. The results demonstrated that *B. amyloliquefaciens* FMME044 is a potential industrial strain for acetoin production.

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1. Introduction

Acetoin is a flavoring compound that is naturally found in fresh apples, milk, wines etc., and widely used in food production and as a chemical raw material and precursor in the synthesis of liquid fuels by microorganisms (Singh and Krishnan, 1959). At present, there are three methods of industrial production of acetoin, including microbial fermentation, enzymatic conversion, and chemical synthesis (Toda et al., 1989). Compared with the chemical method and enzymatic conversion, microbial fermentation is a cost-effective approach with many advantages, including inexpensive raw materials, less environmental pollution, high purity of products, and mild reaction conditions.

It is believed that a high-yielding strain would play a vital role in the commercial fermentation of acetoin. Many microorganisms have the ability to produce acetoin, including *Saccharomyces cerevisiae* (Romano et al., 1993), *Saccharomyces carlsbergensis* (Tomita et al., 1969), *Hanseniaspora guilliermondii* (Teixeira et al., 2002), *Lactococcus lactis* (Bassit et al., 1993), *Lactobacillus casei* (Branen and Keenan, 1971), *Leuconostoc citrovorum* (Collins and Speckman, 1974), and *Zygosaccharomyces bailii* (Romano and Suzzi, 1993). Three other strains, *Serratia marcescens* H13 (75.2 g L^{-1}) (Sun et al., 2012a), *Bacillus licheniformis* MEL 09 (41.2 g L^{-1}), (Liu et al., 2011a), and *Bacillus pumilus* DSM 16187 (63.0 g L^{-1}, EP 2005081882), exhibited high acetoin-producing ability from glucose. In bacteria, much research has focused on the following areas: (1) elucidation of the metabolic control mechanisms (Nicholson, 2008); (2) manipulation of the carbon flux to reduce the production of byproducts (Sun et al., 2012b); (3) isolation of hyper-producing strains (Liu et al., 2011a); and (4) production of highly pure stereoisomeric 2,3-butanediol and acetoin using resting cells (Liu et al., 2011b). In this study, a strain demonstrating a high productivity of acetoin was isolated from the soil and identified as *Bacillus amyloliquefaciens* according to its 16S rDNA

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