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Enhancement of validamycin A production by addition of ethanol in fermentation of *Streptomyces hygroscopicus* 5008

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ABSTRACT

The effect of ethanol on the production of the important agro-antibiotic validamycin A (Val-A) in medium containing agricultural by-products was investigated. Under the optimal condition of ethanol addition, the maximal Val-A production titer reached 18 g/L, which increased by 60% compared to the control. To provide an insight into cell response to ethanol, the intracellular reactive oxygen species (ROS), gene transcription and enzyme activity were determined. Intracellular ROS as the molecular signal was increased in the ethanol condition. Global regulators *afsR* and *glnR* were involved in regulation of Val-A biosynthesis, and the transcription of eight Val-A structural genes was enhanced. The activity of glucose-6-phosphate dehydrogenase (G6PD) was enhanced while glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was inhibited. A signal transduction cascade from cell signal response to activated transcription of Val-A biosynthetic genes and enhanced antibiotic production is proposed. The information can be helpful for the improvement of large-scale fermentation.

1. Introduction

Species in the gram-positive bacterial genus *Streptomyces* synthesize many secondary metabolites, such as germicides, immunosuppressants, pesticides and hydrolases, and more than half of reported antibiotics are produced by *Streptomyces*. Validamycin A (Val-A), called jinggangmycin in China, an extracellular secretion produced by *Streptomyces hygroscopicus* var. *jinggangensis* 5008, is an anti-fungal aminocyclitol antibiotic with high protection efficiency and safety to human and animals. It has been widely used as a primary control reagent against sheath blight disease of rice and wheat plants, false smut of rice and damping-off disease in cucumber and cotton seedlings in East Asia, especially in China. Val-A can be used not only as an agricultural pesticide but also as the synthetic precursor of voglibose and acarbose – two important medicines to treat diabetes (Yu et al., 2005). Due to the wide use of Val-A, the whole gene cluster responsible for Val-A biosynthesis in *S. hygroscopicus* 5008 was cloned, which was composed of eight genes located in three operons, *valABC*, *valKLMN* and *valG* (Bai et al., 2006).

Over the past four decades, attention was paid to enhancing Val-A production by strain screening and medium optimization. Previous reports showed that Val-A fermentation was sensitive to some environmental factors, especially temperature and nonnutritional stresses (Liao et al., 2009). Alcohols can affect secondary metabolism by changing the membrane structure, affecting steady-state growth and regulating related genes and carbon metabolism (Chatterjee et al., 2006). In *Oenococcus oeni*, the presence of ethanol triggers the increase of glutathione reductase, which consumes NADPH, suggesting that maintenance of the redox balance may be related to ethanol adaptation. Additionally, a large increase of dTDT-glucose-4,6-dehydratase and D-alanine:D-alanine ligase, which are known to be involved in cell wall biosynthesis, can also be observed (Silveira et al., 2004). Besides, some citrate pathway genes, such as *citE*, *ackA* and *alsD*, reveal a distinctive transcriptional response in the presence of ethanol (Olguin et al., 2009). Alcohol stress has components common to other stress responses, including metabolite transport and biosynthesis (*malE* and *opp* operon), perturbation of respiratory functions (*nuo* and *cyo* operons), oxidative stress (*sodA*, *sodC* and *yqhD*), and heat shock and cell envelope stress (*rpoE*, *clpB*, *htpG* and *cpxR*) (Rutherford et al., 2010). However, there is little information about the effects of alcohols on intracellular signal, gene expression, enzyme activity in antibiotic biosynthetic pathways, which is of great value to improve antibiotic fermentation technologies and reduce production costs.

In this report, the effects of external ethanol addition on Val-A biosynthesis by *S. hygroscopicus* 5008 were investigated. Different addition concentrations and addition times of ethanol brought a

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