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Short Communication

Enhanced production of ansamitocin P-3 by addition of Mg^{2+} in fermentation of *Actinosynnema pretiosum*

Yongliang Jia [a], Jian-Jiang Zhong [a,b,*]

[a] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China
[b] State Key Laboratory of Microbial Metabolism, and Laboratory of Molecular Biochemical Engineering, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, 800 Dong-chuan Road, Shanghai 200240, China

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ABSTRACT

The effect of divalent metal ions (i.e., Mn^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, and Co^{2+}) on the production of anticancer ansamitocin P-3 (AP-3) by submerged cultures of *Actinosynnema pretiosum* in medium containing agro-industrial residues was investigated, and Mg^{2+} was found to be the most effective. Under the optimal condition of Mg^{2+} addition, the maximal AP-3 production titer reached 85 mg/L, which was 3.0-fold that of the control. The activities of methylmalonyl-CoA carboxyltransferase (MCT) and methylmalonyl-CoA mutase (MCM) were enhanced. The content of two precursors, malonyl-CoA and methylmalonyl-CoA, was lower than that of control. This work demonstrates that Mg^{2+} addition is a simple and effective strategy for increasing AP-3 production through the regulation of enzyme activity and pools of precursors. The information obtained can be helpful to its efficient production on large scale.

1. Introduction

As microelements, metal ions are significant to living system; some of them are even essential components in the survival of organisms. Metal ions also have profound influences on secondary metabolisms, because they may be responsible for the activation of some genes and/or enzymes of biosynthetic pathways (Behal, 1986). Altering the species and concentration of metal ions in the fermentation medium may be useful in enhancing the production of desired metabolites.

Among various metal ions, Mg^{2+} plays an important role in the production of targeted secondary metabolites. For instance, Mg^{2+} has a positive effect on fusarcidins production by *Paenibacillus polymyxa* and geldanamycin production by *Streptomyces hygroscopicus* var. *geldanus* (Wang and Liu, 2008; Raza et al., 2010; Dobson and O'Shea, 2008). Although Mg^{2+} may efficiently improve the biosynthesis of secondary metabolites in some cases, to the best of our knowledge, no study has been conducted on its effect on ansamitocin P-3 (AP-3) production.

The actinomycete *Actinosynnema pretiosum* is of industrial importance because of its production of AP-3, a 19-membered macrocyclic lactam with extraordinary cytotoxic and antineoplastic activities (Ng et al., 2009). Malonyl-CoA and methylmalonyl-CoA are precursors of AP-3, and the sufficient supply of these extender units may enhance AP-3 production (Lin et al., 2011). Methylmalonyl-CoA can be generated by the isomerization of succinyl-CoA by the coenzyme B12-dependent methylmalonyl-CoA mutase (MCM) (Mo et al., 2009), and propionyl-CoA can be synthesized from methylmalonyl-CoA by methylmalonyl-CoA carboxyltransferase (MCT) (Choi et al., 1998). MCM and MCT are two key enzymes in macrolide antibiotic biosynthesis.

In recent years, considerable effort has been focused on enhancing AP-3 production with medium development and genetic modification because of its high commercial value. For example, AP-3 production of 65 mg/L was obtained by the addition of precursor isobutanol (Lin et al., 2011). Meanwhile AP-3 production reached 78 mg/L when the *asm2* deletion mutant was cultivated in an optimized medium (Bandi et al., 2006). Overexpression of *asm2* and *asm39* led to the maximal AP-3 titer of 33 and 52 mg/L, respectively (Ng et al., 2009). As the AP-3 production titer has been not so high, the current study aims to further enhance the accumulation of AP-3 using advanced fermentation technology.

In this work, the addition of Mg^{2+} into the fermentation medium of *A. pretiosum* ATCC 31565 was conducted to enhance AP-3 production. Mg^{2+} concentration and addition time were optimized to improve the antitumor metabolite production. The effect of Mg^{2+} on enzyme activity was further investigated. The information obtained in this work can be helpful in the large-scale production of this valuable bioactive compound.

* Corresponding author at: State Key Laboratory of Microbial Metabolism, and Laboratory of Molecular Biochemical Engineering, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, 800 Dong-chuan Road, Shanghai 200240, China. Tel.: +86 21 34206968; fax: +86 21 34204831.
E-mail address: jjzhong@sjtu.edu.cn (J.-J. Zhong).

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Table 1
Effect of addition of different metal ions on AP-3 production.[a]

Metal ions	DCW (g/L)	AP-3 (mg/L)
Mn^{2+} (2.0 mM)	14.5 ± 0.79	22.46 ± 0.75
Mn^{2+} (5.0 mM)	15.02 ± 0.88	34.97 ± 1.00
Mg^{2+} (0.5 mM)	15.72 ± 0.58	43.07 ± 6.22
Mg^{2+} (2.0 mM)	16.52 ± 0.58	72.64 ± 3.19
Zn^{2+} (0.1 mM)	14.12 ± 0.98	18.52 ± 0.82
Zn^{2+} (0.5 mM)	13.43 ± 0.35	20.52 ± 0.97
Zn^{2+} (2.0 mM)	13.30 ± 0.67	33.61 ± 1.89
Cu^{2+} (0.05 mM)	13.70 ± 0.76	18.13 ± 2.01
Cu^{2+} (0.1 mM)	13.18 ± 1.08	17.27 ± 1.92
Cu^{2+} (0.5 mM)	11.85 ± 0.15	14.51 ± 1.61
Co^{2+} (0.1 mM)	11.47 ± 0.20	20.07 ± 0.78
Co^{2+} (0.5 mM)	10.20 ± 0.69	12.37 ± 0.46
Co^{2+} (2.0 mM)	9.05 ± 0.88	7.78 ± 0.29
Control	14.57 ± 0.77	21.49 ± 1.07

[a] Fermentation medium consisted of (in g/L, pH 7.45) glucose 5, yeast extract 10, glycerol 40, buckwheat filtrate 20, $CaCO_3$ 5, K_2HPO_4 0.5, and $FeSO_4{\cdot}7H_2O$ 0.002. A different concentration of metal ions (Mn^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}) was added as sulfate salts to autoclaved fermentation medium, with an untreated culture as the control. The flasks (60/250 mL) were incubated at 28 °C for 192 h. The cells were harvested at 192 h and then DCW and AP-3 production were analyzed.

2. Methods

2.1. Microorganism and culture conditions

A. pretiosum ATCC 31565 was used in this study. Detail on the culture medium and procedures of inoculation were reported earlier (Lin et al., 2011). Different concentrations of metal ions (Mn^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}) were added aseptically as sulfate salts to autoclaved fermentation medium, with an untreated culture serving as the control.

2.2. Growth measurement and ansamitocin P-3 (AP-3) determination

Biomass accumulation was estimated using dry cell weight analysis (Lin et al., 2011). AP-3 was extracted and determined as described (Lin et al., 2011).

2.3. Determination of extracellular and intracellular Mg^{2+} concentration

Magnesium ion concentration was measured with inductively coupled plasma emission spectrometer (Thermo fisher, iCAP6300, USA) using standard procedures.

2.4. Detection of malonyl-CoA, methylmalonyl-CoA and enzyme activity

Malonyl-CoA and methylmalonyl-CoA were extracted and determined as described (Lin et al., 2011).

Activities of methylmalonyl-CoA carboxyltransferase (MCT) and methylmalonyl-CoA mutase (MCM) were determined as reported (Choi et al., 1998; Du and Chen, 2007).

3. Results and discussion

3.1. Effect of different metal ions on AP-3 production

According to our preliminary experiments plus reference information (e.g., Raza et al., 2010; Weinberg, 1990), the particular concentration range of a given metal ion was chosen. For example, our preliminary experiments indicated that 5 mM Co^{2+} or Cu^{2+} completely inhibited the cell growth; therefore we reduced their concentration range to a lower level. As shown in Table 1, only the addition of Mg^{2+} resulted in the significant enhancement of AP-3 production with maximum titer of 72.64 mg/L, and the dry cell weight (DCW) slightly increased after 192 h of cultivation. Compared with the salts of Mg^{2+}, those of Mn^{2+} (at 5 mM) and Zn^{2+} (at 2 mM) only slightly improved the AP-3 production. In contrast, Cu^{2+} and Co^{2+} were toxic and suppressed both the growth of *A. pretiosum* and AP-3 production.

Metal ions are necessary for the normal metabolism of microorganisms as microelements as well as for the production of secondary metabolites (Milner et al., 1995). Results reveal that Mg^{2+} caused the significant enhancement of AP-3 production.

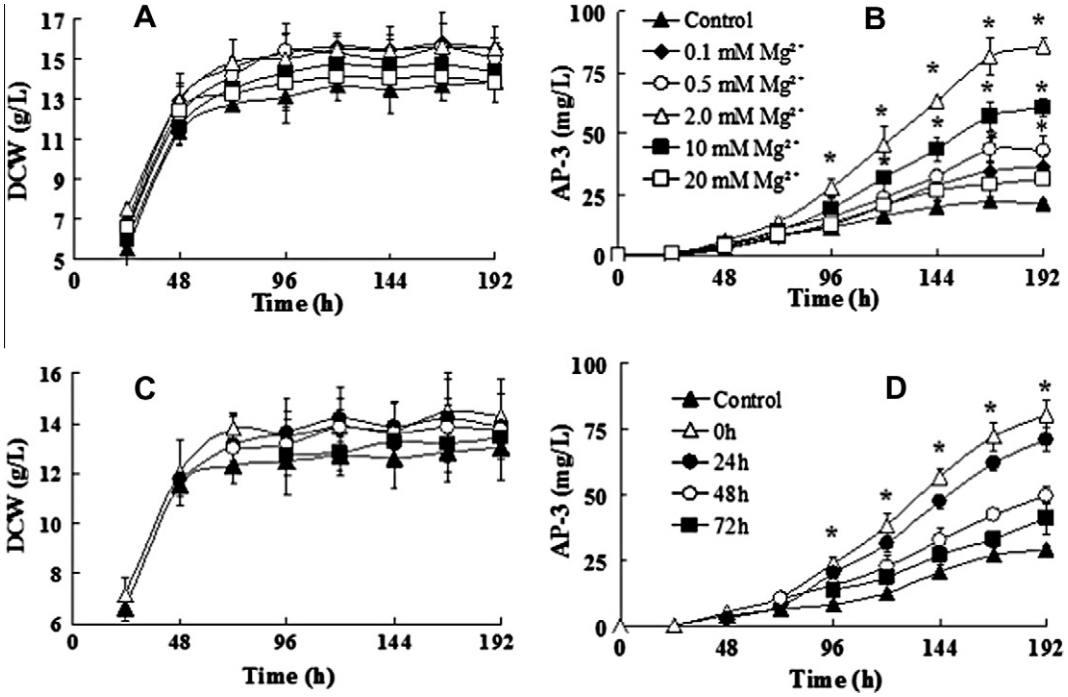

Fig. 1. Effect of Mg^{2+} addition concentration and time on *A. pretiosum* cell growth and AP-3 production. (A) DCW when different concentration of Mg^{2+} was added at 0 h (inoculation time); (B) AP-3 production when different concentration of Mg^{2+} was added at 0 h; (C) DCW when 2 mM Mg^{2+} was added at different time; (D) AP-3 production when 2 mM Mg^{2+} was added at different time. Each data point represents the mean ± SD from three independent samples, $^{*}p < 0.05$.