

Full Length Research Paper

Effect of propionate on the production of natamycin with *Streptomyces gilvosporeus* XM-172

Keju Jing*, Xiaobin Hao and Yinghua Lu

Department of Chemical and Biochemical Engineering, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, P. R. China.

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This study described the influence of feeding short-chain fatty acids and alcohols on natamycin production in the glucose basal medium, produced by *Streptomyces gilvosporeus* XM-172. The highest natamycin production was obtained with feeding propionate as compared to other precursors. The optimal propionate concentration and feeding time were 6 g L⁻¹ and early log phase, respectively. This optimal propionate feeding strategy led to a natamycin production of 6.72 g L⁻¹, which was nearly 85% higher than that of the control. It was firstly revealed that propionate could greatly promote natamycin biosynthesis by *S. gilvosporeus*.

Key words: Precursor, propionate, natamycin production, *Streptomyces gilvosporeus* XM-172.

INTRODUCTION

Natamycin is an important polyene macrolide antifungal antibiotic produced by microorganisms from some genus *Streptomyces* strains such as *Streptomyces natalensis*, *Streptomyces chattanoogensis* and *Streptomyces gilvosporeus* (El-Enshasy et al., 2000). This antibiotic can effectively inhibit the growth of both yeasts and molds (Pedersen, 1992) and prevent the aflatoxin formation in filamentous fungi (Aparicio, 2004). Because of its wide spectrum activity against both yeast and molds with low toxicity against mammalian, natamycin has been widely used in food preservation to increase the shelf time of many food products (Cong et al., 2007; Delves-Broughton et al., 2006). It is one of the few antibiotics recommended by FDA as food additive and classified as a GRAS (generally regarded as safe) compound. Besides its food applications, natamycin can also be applied in the treatment of many fungal diseases such as bronchopulmonary aspergillosis (Patterson and Strek, 2010) and mycotic keratitis (Malecha, 2004).

Natamycin represents a typical molecule of the 26-membered ring macrocyclic polyketides, which are synthesized by a common pathway where units derived from acetate, propionate or butyrate are condensed onto

the growing chain by a polyketide synthase (PKS) in a process that resembles the long-chain fatty acid biosynthesis (Staunton, 1991; Hopwood, 1997). Although, acetate and propionate are the immediate precursors for formation of polyene macrolide antibiotics, they do not support antibiotic synthesis. However, supplementation of a glucose basal medium with acetate or propionate produces a significant stimulation of candicidin and fungimycin biosynthesis in batch cultures (Martin and Liras, 1976; Martin, 1977). Similar to the biosynthesis of other polyene macrolide antibiotic, it might facilitate natamycin biosynthesis by feeding precursors of short-chain fatty acids and alcohols in the glucose culture medium.

Due to the important commercial value of natamycin, it is necessary to improve the productivity of natamycin by strain improvement or bioprocess optimization. Until now, some studies focused on the influence of different medium constituents and fermentation conditions on the production of natamycin (Liang, 2008; Chen, 2008). Few data are available on the influence of precursors of short-chain fatty acids and alcohols on the biosynthesis of natamycin by *S. gilvosporeus* strains. In this study, influence of different precursors derived from acetate, propionate or butyrate on natamycin biosynthesis by *S. gilvosporeus* strains was investigated. When the optimal precursor was determined, the effect of precursor

*Corresponding author. E-mail: jkj@xmu.edu.cn.

Table 1. Effect of various precursors on biomass and natamycin production.

Precursor	Concentration (g / L)	Natamycin (g/L)		DCW ^a (g/L)		Yield ^b (g/g)	
		Value	Increase (%)	Value	Increase (%)	Value	Increase (%)
Control	0	3.63	-	29.11	-	0.125	-
Isobutyl alcohol	1	4.32	19.0	31.27	7.42	0.138	10.4
Ethanol	1	4.53	24.8	30.38	4.36	0.149	19.2
n-butyl alcohol	1	4.67	28.7	30.98	6.43	0.152	21.6
Sodium acetate	1	5.20	43.3	32.85	12.85	0.158	26.4
Sodium propionate	1	5.64	55.4	33.23	14.15	0.170	36.0

^a Mycelial dry weight; ^b Yield of product from the biomass (g of product per g of mycelial dry weight). All the experiments were carried out at 28°C and 220 rpm with addition of 1 g/L precursors into 40 ml production medium in 500 ml Erlenmeyer flask at the early log phase of 120 h of cultivation. The concentration of natamycin in the broth was determined by HPLC from the literature (Chen et al., 2003). The production medium was composed of (g/L): glucose, 60; soybean cake meal, 10.0; peptone, 5.0; yeast extract, 5.0; beef extract, 5.0; NaCl, 2.0; CaCO₃, 5.0; MgSO₄, 1.0 and pH 7.6 before sterilization. The values were the mean ± S.E.M of three separate experiments.

concentration and feeding time was further observed.

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MATERIALS AND METHODS

S. gilvosporeus XM-172, a high-yield mutant derived from *S. gilvosporeus* ATCC 13326, was used in this study.

Media and culture conditions

Slant medium (g/L): Glucose, 10.0; malt extract, 3.0; yeast extract, 3.0; peptone, 5.0 and agar, 20.0. The pH of the medium was pre-adjusted to 7.2 to 7.4 before sterilization. Slant culture was incubated for 6 to 7 days at 28°C and 30 to 60% humidity.

The spores from slant culture were inoculated into a 500 ml Erlenmeyer flask containing 40 ml of seed medium (glucose, 20.0 g/L; malt extract, 6.0 g/L; peptone, 6.0 g/L; NaCl, 0.2 g/L and pH 7.0 to 7.2 before sterilization). After incubation at 28°C for 48 h on a rotary shaker at 220 rpm, a 5 ml portion of the seed culture was used to inoculate 40 ml production medium in 500 ml Erlenmeyer flask. The production medium was composed of (g/L): glucose, 60; soybean cake meal, 10.0; peptone, 5.0; yeast extract, 5.0; beef extract, 5.0; NaCl, 2.0; CaCO₃, 5.0; MgSO₄, 1.0 and pH 7.6 before sterilization. The production culture was incubated under the same conditions as the seed culture, except the investigated conditions.

Determination of mycelial dry weight

A 3 ml aliquot of the fermentation broth was periodically sampled to determine the cell dry weight. The sample was centrifuged at 3000 rpm for 5 min, and the cell pellet was washed with distilled water twice. Cell dry weight was determined by filtering the fluid through a pre-weighed filter paper (Whatman GF/C), which was dried to constant weight at 80°C in a vacuum oven.

Determination of natamycin

Natamycin analysis was performed on a Biotronik HPLC system (Maintal, Germany), equipped with a Phenomenex prodigy ODS3 100 A column (5 µm, 250 × 4.6 mm) (Phenomenex Australia, Lane Cove, NSW, Australia). The mobile phase composition was methanol-water-phosphoric acid (85:15:0.15 v/v/v) and set at a flow rate of 1 ml/min. Detection of natamycin was performed at 303 nm and quantified with an external standard (Sigma-Aldrich, St. Louis,

RESULTS AND DISCUSSION

Acetate, propionate or butyrate as the immediate precursors is involved in macrolide chain of natamycin biosynthesis, which mainly comes from intermediates production of primary metabolism in batch fermentation. When these short-chain fatty acids are not required in biosynthesis of the macrolactone ring of natamycin, they become important in rate-limiting step of natamycin biosynthesis (Bibb, 2005).

Based on previous study of natamycin biosynthesis metabolism pathway (Aparicio et al., 2000), several short-chain fatty acids and alcohols were selected as precursor and they were added to the glucose basal culture, and the effects of these precursors on the natamycin production and biomass by *S. gilvosporeus* XM-172 were investigated. As shown in Table 1, same concentration (1 g/L) of precursors, including ethanol, isobutyl alcohol, n-butyl alcohol, sodium acetate and sodium propionate, were fed in the production medium at the early log phase, respectively. These precursors could both promote mycelial growth and natamycin production as compared to the control (Table 1). The results show that natamycin biosynthesis was facilitated by feeding short-chain organic acids and alcohol. For better understanding, the relation of mycelial growth and natamycin production, and the natamycin yield based on biomass [$Y_{p/x}$] was calculated.

The maximal efficiency of precursors for natamycin production and yield were as followings: sodium propionate > sodium acetate > n-butyl alcohol > ethanol > isobutyl alcohol. Among them, the optimal precursor was sodium propionate. The highest natamycin production reached 5.64 g/L and increased to 55.4% as compared to the control.

Therefore, propionate was considered as the optimal precursor in this study. Once propionate is determined to

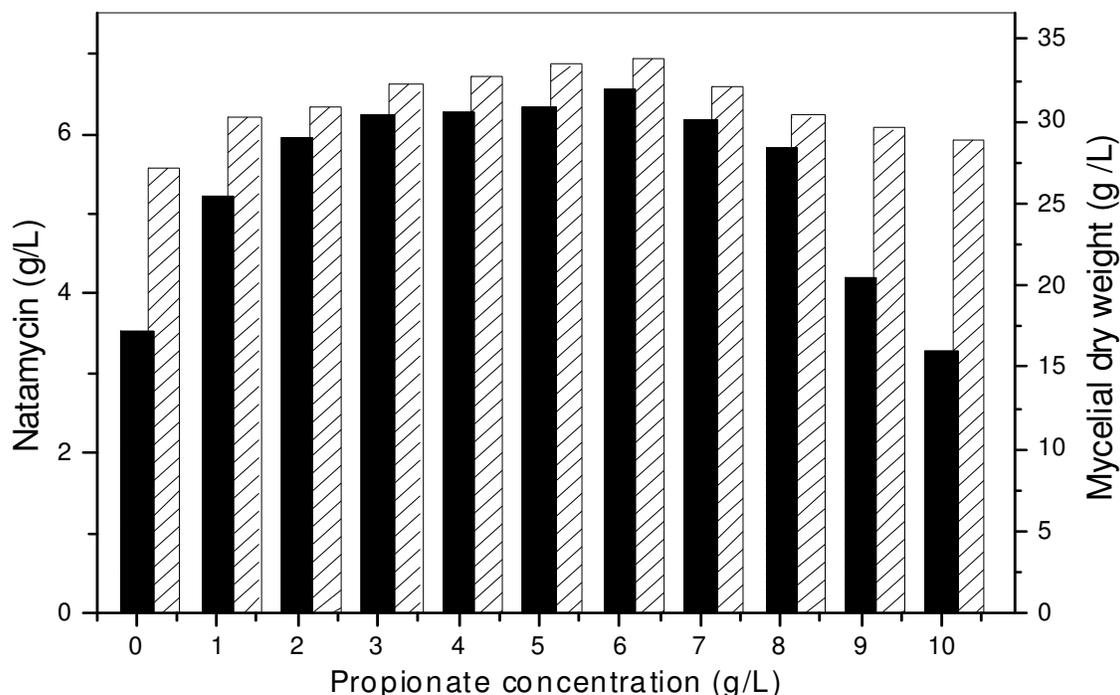


Figure 1. Effect of propionate concentration on mycelial dry weight and natamycin production. All the experiment were carried out at 28°C and 220 rpm with addition of different propionate concentration to 40 ml production medium at early log phase for 120 h of cultivation. Black bar chart is natamycin production and sparse bar chart is mycelial dry weight.

Table 2. Effect of propionate feeding time on mycelial growth and natamycin production in production medium.

Parameter	F0	F0	F1	F1	F2	F2	F3	F3
Propionate feeding time (hour)	0	12	24	36	48	60	72	84
Natamycin production (g L ⁻¹)	6.34	6.42	6.72	6.51	6.17	6.04	5.08	4.21
Mycelia dry weight (g L ⁻¹)	33.87	33.58	33.27	34.86	34.57	31.35	30.56	29.71
Natamycin yield (g g ⁻¹)	0.187	0.191	0.202	0.187	0.178	0.192	0.166	0.141
Natamycin productivity (g d ⁻¹ L ⁻¹)	1.27	1.28	1.34	1.30	1.23	1.21	1.02	0.84

*F0: the lag phase; F1: the early log phase; F2: the late log phase; F3: the stationary phase. The experiment was carried out at 28°C and 220 rpm by adding 6 g/L propionate to 40 ml production medium at different feeding time of 120 h of cultivation.

have significant influence on natamycin biosynthesis, it is necessary to investigate whether natamycin production could be further improved by adjusting propionate concentration in the batch culture. For this purpose, different concentrations of propionate, ranging from 1 to 10 g/L, were added to fermentation broth to evaluate the effect on mycelial growth and natamycin production. As shown in Figure 1, both mycelial dry weight and natamycin production reached maximal values with 6 g/L propionate after 120 h of cultivation. The highest natamycin production reached 6.54 g/L, which was 75% more than that of the control. When propionate concentration increased from 1 to 6 g/L, the biomass and natamycin production increased. Above 6 g/L propionate, the biomass and natamycin production decreased with propionate concentration further increasing. It could be

explained that high concentration of propionate resulted in substrate-inhibition on natamycin biosynthesis, and inhibition of mycelial growth due to rising pH of fermentation broth. Therefore, the optimal propionate concentration was 6 g/L in this study.

Natamycin production reached a maximal value at 120 h of cultivation process. In order to further improve natamycin production of *S. gilvosporeus* XM-172, the effect of feeding propionate on different growth stages was investigated. The propionate was fed in the production medium with an initial concentration of 6 g/L at one of the four phases during the culture: (1) the lag phase, (2) the early log phase, (3) the late log phase and (4) the stationary phase. Detailed information of propionate feed timing is listed in Table 2. In the experimental results, both the biomass and natamycin

production increased before propionate feeding at the stationary phase. The highest natamycin production (6.72 g/L) was obtained in the culture with feeding of 6 g/L propionate at the early log phase. Propionate fed at the stationary phase could not increase the biomass and natamycin production as compared to the control. It might be explained that propionate could not be effectively converted into propionyl-CoA, the precursors for macrolide biosynthesis by *S. gilvosporeus*, due to correlative enzyme activities dramatically decreasing at the station phase as compared to the early log phase (the onset of natamycin biosynthesis phase) (Bibb, 2005).

Conclusions

The natamycin production in *S. gilvosporeus* XM-172 could be significantly increased by feeding propionate in cultivation process. In this study, the propionate was superior to other precursors on promoting natamycin biosynthesis. The optimal propionate concentration and feeding time were 6 g/L and 24 h, respectively. Under the optimal condition, natamycin production reached 6.72 g/L and increased to 85% as compared to the control. It was firstly found that the addition of propionate to the fermentation broth could enhance natamycin biosynthesis with *S. gilvosporeus*.

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