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RESEARCH PAPER

A pH Shift Feeding Strategy for Increased Enduracidin Production During Fed–batch Fermentation by a Deep–sea, Bacterium, *Streptomyces* sp. MC079

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Abstract The aim of this work is to enhance enduracidin production by *Streptomyces* sp. MC079. Based on the time course analysis of the specific cell growth rate and specific enduracidin formation rate, a two–stage pH control strategy was proposed to improve enduracidin production by shifting the culture pH from 5.5 to 5.8 after 112 h of cultivation. By applying this pH control strategy, enduracidin concentration and productivity was 51.2 and 65.0% higher than results with uncontrolled pH batch fermentation. For further enhancement of enduracidin production, the effects of constant–rate feeding and pH-shift feeding strategy were investigated. The results indicated that the pH-shift feeding strategy increased the maximum concentration and productivity of enduracidin to 61.37 mg/L and 0.697 mg/L/h in the constant–rate feeding fermentation process. This is 73.3 and 88.9% higher than results with uncontrolled pH batch fermentation, respectively. The obtained optimal pH shift feeding strategy may be useful for the industrial–scale microbial production of enduracidin.

Keywords: *Streptomyces* sp. MC079, enduracidin, pH-shift feeding strategy, fed–batch fermentation

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1. Introduction

Enduracidin, first isolated by Takeda Chemical Industries Ltd. of Japan, is a polypeptide antibiotic produced by *Streptomyces* composed of unsaturated fatty acids and ten different of amino acids [1]. It is comprised of two main components: enduracidin A and enduracidin B [2]. Its molecular weight is about 2,500 with a melting point between 238 and 245°C. It is soluble in dilute hydrochloric acid, slightly soluble in water, methanol, and ethanol, and insoluble in acetone, benzene, and chloroform. Enduracidin has strong stability under heat, light and moisture conditions [3]. It is stable in a pH range of 3.5 ~ 7.5 for 3 days at 25°C. Conversely, when the pH is lower than 3.0 or higher than 9.0, it is easily to degraded. Enduracidin is an excellent polypeptide antibiotic due to its effective low dosage, low residue, unlikelihood to produce drug resistance, powerful bactericidal effects on most Gram–positive bacteria, and ability to promote animal growth. Lastly, Miyake has found that the acid salt of enduracidin is stable and shows strong antibacterial effect [4].

There are several reports concerning the microbial bioprocesses and various strains for enduracidin production. Matsumura established a method for screening high-yield enduracidin strains, as well as, studied the resulting enduracidin composition and structure [5]. A novel enduracidin producing strain, *Streptomyces atrovirens* (CGMCC No.3367), was discovered through genetic screening methods [6]. An enduracidin biosynthesis strain, *Streptomyces fungicidicus*, was isolated from soil in JAPAN [7]. A new enduracidin producer, *Streptomyces atrovirens* MGR140, was identified and confirmed by gene disruption and HPLC analysis [8]. Using the halogenase gene as a screening

probe, Xiao *et al*. have identified an enduracidin producing bacterium, *Streptomyces atrovirens* Z40, whose halogenase gene is highly homologous to the halogenase gene involved in enduracidin biosynthesis in 163 *actinomycetes* isolated from mangrove soil [9].

Previous research has established that glucose and starch are the best carbon sources for enduracidin production by *Streptomyces atrovirens* Z40 [10]. Using single–factor and orthogonal optimization, enduracidin production was increased by 360% than using basic medium. Fermentation is significantly influenced by various physical and chemical parameters; among which pH is regarded as one of the most important factors since it can affect the duration and rate of fermentation, as well as, the production of fermentation metabolites [11]. Many studies have used a pH control strategy successfully to improve fermentation yields. Xu [12] proposed a two-stage pH control strategy for the production of rhamsan gum. Luo [13] suggested a two-stage pH control strategy which the pH was maintained at 7.0 for the first 24 h and then shifted to 5.0 after 24 h for increased β-carotene production. A novel two-stage pH control strategy was developed to improve ε-poly-L-lysine production by shifting the culture pH from 3.5 to 3.8 after 36 h of cultivation [14]. An alkaline pH control strategy was developed to improve methionine adenosyltransferase production in *Pichia pastoris* fermentation [15]. However, there are no studies documenting the use of a pH control strategy to improve the production of enduracidin.

In this study, we explored the effects of pH and glucose feeding strategies on enduracidin production. Our results show that the use of a pH-shift feeding strategy can significantly increase the concentration of enduracidin, up to 61.37 mg/L. The results presented here may be useful for industrial–scale production of enduracidin.

2. Materials and Methods

2.1. Microorganism

Streptomyces sp. MC079 (MCCC 1A02705) was isolated from deep–sea sediment in the eastern Pacific Ocean at a depth of 5,143 m.

2.2. Media And culture conditions

The agar slant medium contained: 20 g/L glucose, 3 g/L yeast extract, 2 g/L peptone, 0.1 g/L $FeSO_4$, 2 g/L K_2HPO_4, 10 g/L NaCl, and 20 g/L agar at pH 7.2 to 7.4. The seed medium contained: 20 g/L glucose, 3 g/L yeast extract, 2 g/L peptone, 0.1 g/L $FeSO_4$, 2 g/L K_2HPO_4, and 10 g/L NaCl at pH 7.2 ~ 7.4. The medium for enduracidin fermentation contained: 40 g/L glucose, 5 g/L peptone, 0.1 g/L $FeSO_4$, 2 g/L K_2HPO_4, and 10 g/L NaCl with the initial pH of fermentation medium adjusted to 7.4.

The strain was preserved on agar slants and incubated at 28°C for 4 days. After growth and sporulation, one loopful of *Streptomyces* sp. MC079 spores was transferred to 50 mL seed medium in a 250 mL shake flask. The strain was incubated at 28°C and agitated at 180 rpm for 40 h.

Batch fermentation was carried out in a 6.6 L stirred fermenter (Biostat 5, Sartorius, Germany) in 5 L of working volume. The seed culture (5%, v/v) was inoculated into the fermentation medium. Cultures were agitated by two Rushton impellers and varied from 200 to 800 rpm. Aeration was adjusted by a ring sparger with a range of 1.0 ~ 2.5 L/L/min, rendering dissolved oxygen (DO). DO content was constantly monitored and maintained at approximately 20% with a DO electrode (Mettler-Toledo International, Inc. Ohio, USA). During cultivation, the pH changes were detected by a pH electrode (Mettler-Toledo International, Inc.). The fermentation temperature was maintained at 28°C by a re–circulating water bath.

2.3. Effect of pH on enduracidin production in batch fermentation

To investigate the effect of pH on enduracidin production, *Streptomyces* sp. MC079 was cultivated in fermentation medium in a stirred fermenter at an initial pH of 7.4 at a final broth volume of 3.3 L. During the cultivation, when pH dropped from the initial pH of 7.4 ~ 5.5, the pH was controlled at 5.3, 5.5, 5.8, 6.0, or 6.2 by automatically adding 12.5% (v/v) NH_4OH solution or a phosphoric acid solution to the culture broth until the end of cultivation.

2.4. Two–stage pH control strategy for enduracidin production in batch fermentation

Based on the specific cell growth rate and specific enduracidin formation rate, a two–stage pH control strategy was proposed as follows: when pH decreased from initial 7.4 to 5.5, the pH was controlled at 5.5 for 112 h of cultivation, and then pH was stepwise shifted to 5.8 to further promote enduracidin synthesis in late cultivation.

2.5. A Constant–rate feeding strategy for enduracidin production in fed–batch fermentation

A constant–rate feeding strategy in fed–batch culture was employed with an initial glucose concentration of 14 g/L. The initial broth volume for fed–batch culture was 3.0 L. When the residual glucose concentration dropped under 5 g/L, feeding of concentrated glucose (300 g/L) was initiated at the feeding rate of 6.25 mL/h by a computer–controlled pump. Total feeding volume was 300 mL.

2.6. Analytical methods

Samples were withdrawn from fermenter for analysis at

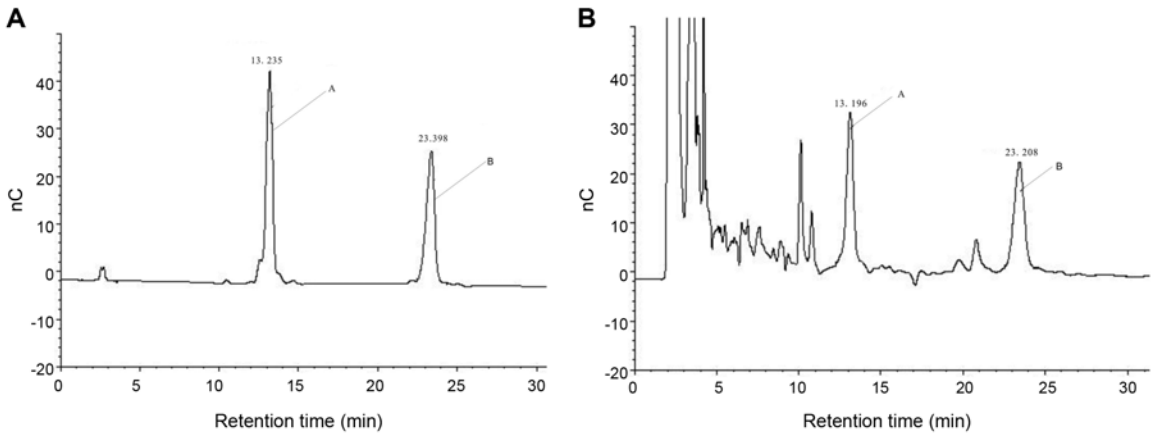

Fig. 1. HPLC chromatogram of enduracidin, (A) reference substance for enduracidin, (B) enduracidin from fermentation product.

Table 1. Effect of initial glucose concentration on cell growth and enduracidin production

Parameters	Initial glucose concentration (g/L)						
	20	30	40	50	60	70	80
Residual glucose concentration (g/L)	0	0	0.12	10.85	21.07	32.33	45.57
DCW (g/L)	6.21	7.22	7.35	7.87	7.51	5.11	3.74
Enduracidin concentration (mg/L)	18.46	26.23	35.42	32.13	24.58	16.71	8.47

regular intervals. The biomass was determined in triplicate for 50 mL cell suspensions that were harvested by centrifugation (6,000 × *g*, 5 min), washed with distilled water, and then dried at 60°C for 24 h to a constant weight (dry cell weight, DCW). Glucose concentration was measured with a Bioprofile 300A biochemical analyzer (Nova Biomedical, MA, USA).

The dry cell was–resuspended in 90% methanol solution, and then broken by an ultrasonic wave. The supernatant was used to determine the amount of enduracidin by HPLC (U–3000, Dionex, Thermo Fisher Scientific, USA), using a C_{18} reverse phase chromatography column (250 mm × 2.1 mm, Hercules, CA, USA). The mobile phase was acetonitrile–50 mM sodium dihydrogen phosphate (3:7, v/v) with the pH adjusted by a phosphoric acid solution to 4.5. Before use, the mobile phase was filtered with 0.22 μm microfiltration membrane. Sample injection volume was set to 20 μL and the column was eluted at 25°C with a flow rate of 1.0 mL/min. Fig. 1 displays the resulting chromatogram of enduracidin.

3. Results and Discussion

3.1. Effects of initial glucose concentration on enduracidin production

To study the effects of glucose concentration on cell growth and enduracidin production in *Streptomyces* sp. MC079, various glucose concentrations (from 20 to 80 g/L) with the same carbon/nitrogen ratio (C/N) were tested (Table 1). When glucose concentration was within 30 ~ 60 g/L, the final biomass was approximately the same (between 7.2 and 7.5 g/L DCW) for each batch. However, the final biomass declined in the presence of 70 or 80 g/L glucose, indicating that a high glucose concentration was unfavorable for cell growth. Further, addition of 40 g/L initial glucose led to the maximum enduracidin production of 35.42 mg/L at 160 h. When the initial glucose concentration was 80 g/L, both cell growth and enduracidin production were significantly inhibited (Table 1), and the residual concentration of glucose remained at 45.57 g/L even after 168 h of cultivation. These results showed that enduracidin production was more efficient at an appropriate glucose concentration of 40 g/L.

3.2. Time profiles of enduracidin fermentation by *Streptomyces* sp. MC079 in batch fermentation

The typical fermentation process of *Streptomyces* sp. MC079 without pH control is shown in Fig. 2. The pH of the fermentation broth decreased from its initial value of 7.4 ~ 5.31 within the first 72 h of fermentation, but the pH did not change much afterward. Before the pH decreased to 5.31, the cell growth increased rapidly and reached the maximum of 7.35 g/L at 72 h of cultivation. However, after the cell growth reached the maximum, a decrease of mycelia growth was observed. This result may be due to

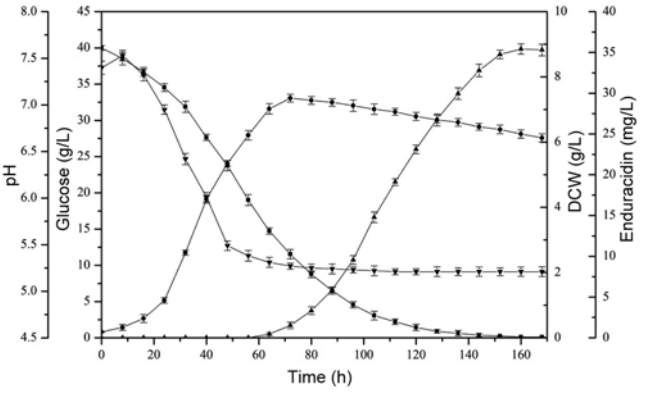

Fig. 2. Time profiles of cell growth, enduracidin concentration, glucose concentration and pH during batch fermentation of *Streptomyces* sp. MC079 for enduracidin production. Enduracidin (▲), DCW (●), pH (▼), Glucose (■).

the low pH of the fermentation medium. The enduracidin accumulation started when the pH was less than 6.0, and reached the maximum of 35.42 mg/L at 160 h. Eventually, enduracidin accumulation stopped after 160 h of cultivation. As cell growth increased, glucose was consumed during the initial 72 h of fermentation, while the enduracidin accumulated from 64 to 160 h during fermentation. The concentration of glucose was decreased from its initial concentration of 40.0 to 0.1 g/L after 168 h of cultivation. We deduced from these observations, that the pH of the culture broth is critical for cell growth and enduracidin production. Thus, by proper control of culture broth pH, cell growth may be prolonged and enduracidin production enhanced.

3.3. Effects of pH on enduracidin production by *Streptomyces* sp. MC079 in batch fermentation

Effects of pH on different acidification products and types of fermentation have been studied in different fields; for example, acidogenic fermentation of fruit and vegetable wastes [16]. The effects of varying pH values on enduracidin production fermentation by *Streptomyces* sp. MC079 were investigated (Fig. 3). During the cultivation, when the pH decreased from initial 7.4 to 5.5, the pH was controlled at 5.3, 5.5, 5.8, 6.0, and 6.2 until the end of cultivation. At pH levels below 5.5 or above 6.0, enduracidin production was low. Because of the low enduracidin production at the afore mentioned pH levels, the time–course data of these cultures and the related kinetic parameters is not presented here.

As shown in Fig. 3A, cell growth increased when pH

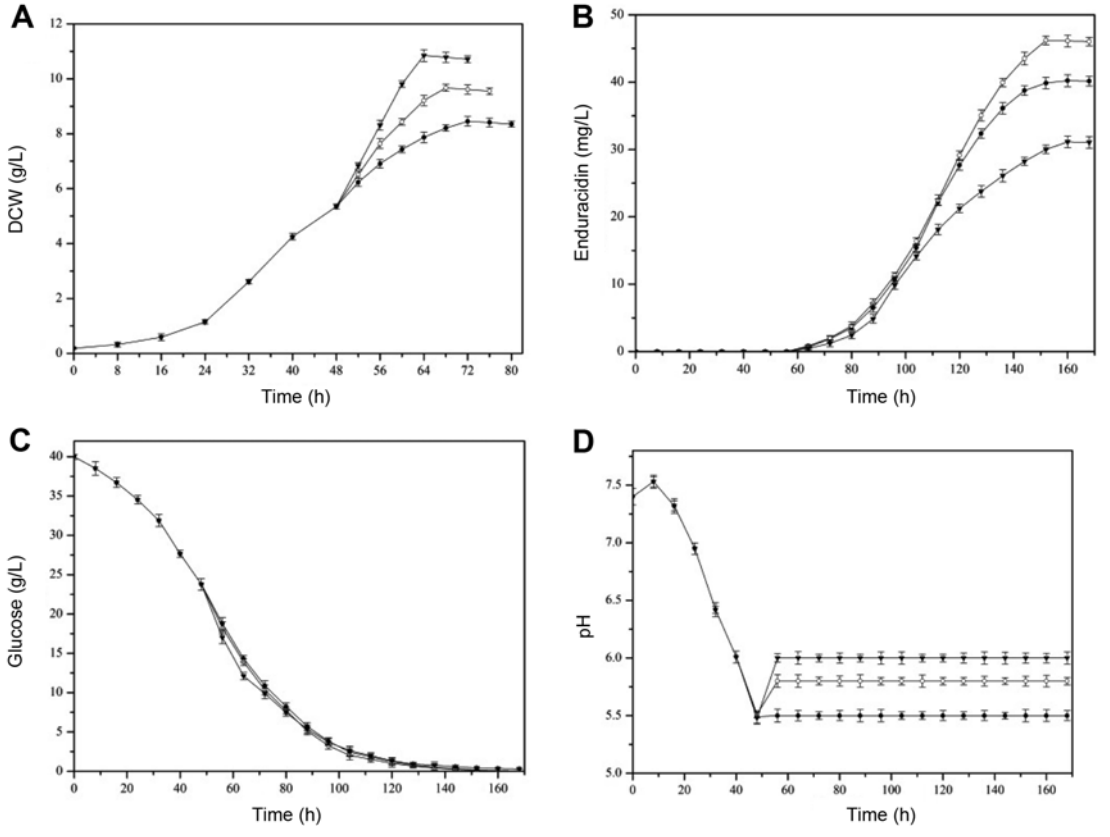

Fig. 3. Time profiles of cell growth (A), enduracidin concentration (B), glucose concentration (C) and pH (D) during cultivation of *Streptomyces* sp. MC079 at different pHs for enduracidin production. pH 5.5 (●), pH 5.8 (○), pH 6.0 (▼).

was increased from 5.5 to 6.0. With the increase of pH, the time to reach the stationary phase decreased as the cell concentration increased. When the pH was set at 6.0, DCW reached a maximum of 10.85 g/L at 64 h. Fig. 3B shows that the relatively higher enduracidin concentrations of 40.23 and 46.21 mg/L were obtained at the pH of 5.5 and 5.8, respectively. These data suggest that the production of enduracidin by *Streptomyces* sp. MC079 was affected by pH, and that neither low (5.3) nor high (6.0) pH is beneficial for enduracidin production. Consistent with the increase of cell growth, the consumption of glucose was increased from pH 5.5 to 6.0 (Fig. 3C). After the biomass reached the maximum DCW, the consumption of glucose decreased. Glucose was exhausted at 152 h (pH 5.8) which was increased in comparison to glucose consumption under pH 5.5 and pH 6.0 conditions

3.4. Kinetic analysis of enduracidin fermentation at different pH values

To analyze the kinetic characteristics of pH effects on cell growth and enduracidin production, specific cell growth rate (μ_x, /h) and specific enduracidin formation rate (q_p, /h) (mg/h/g DCW) were calculated using the data in Fig. 3. Figs. 4A and 4B show that when a lower pH is maintained, μ_x and q_p decrease rapidly after pH is controlled at the set values. Before 112 h of cultivation, the q_p iwas the highest under pH controlled at 5.5. After 112 h of cultivation, the q_p iwas the highest under pH controlled at 5.8. Therefore, to improve the efficiency of enduracidin production, it is presumed that enduracidin accumulation in fermentative system by *Streptomyces* sp. MC079 is separately regulated: pH 5.5 was preferred in the early stage of cultivation (before 112 h) to ensure a higher specific enduracidin formation rate, and pH 5.8 was then required after 112 h to ensure enduracidin formation.

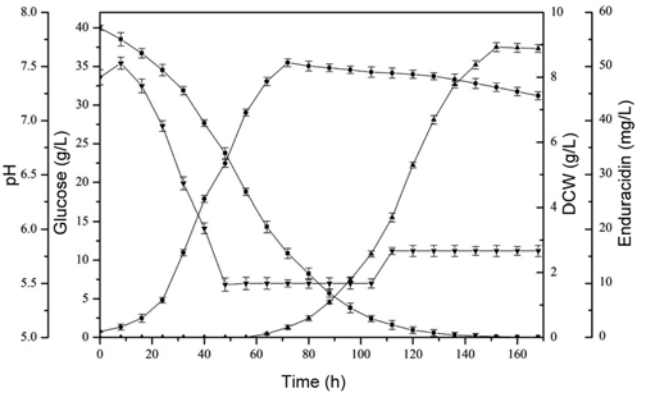

Fig. 5. Time profiles of cell growth, enduracidin concentration, glucose concentration and pH during batch fermentation of *Streptomyces* sp. MC079 using the two–stage pH control strategy with pH shifted from 5.5 to 5.8 at 112 h of cultivation. Enduracidin (▲), DCW (●), pH (▼), Glucose (■).

3.5. Batch fermentation for enduracidin production with two–stage pH control strategy by *Streptomyces* sp. MC079

Based on the analysis of μ_x, and q_p, an optimal two–stage pH controlled strategy was developed to optimize enduracidin production. When the pH naturally decreased from the initial pH of 7.4 ~ 5.5, culture pH was controlled at pH 5.5 until 112 h of cultivation, and then pH was shifted to 5.8 to further promote enduracidin formation in later fermentation. The time course of two–stage pH controlled strategy for enduracidin fermentation is shown in Fig. 5. The maximum concentration and productivity of enduracidin reached 53.57 mg/L and 0.609 mg/L/h in the two–stage pH controlled fermentation process, which is 51.2 and 65.0% higher than the results with pH uncontrolled batch fermentation, respectively (Table 2). The two–stage pH control strategy not only considerably improved enduracidin production

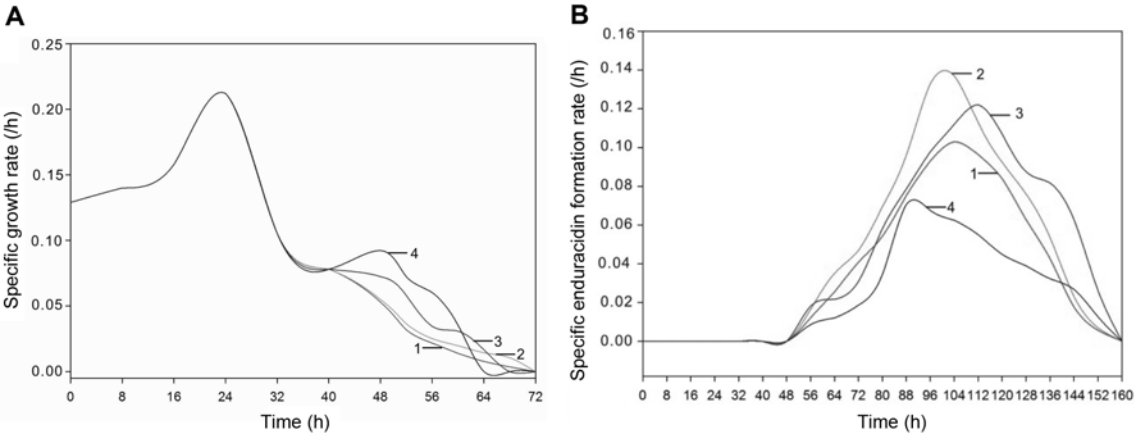

Fig. 4. Time profiles of specific growth rate (A) and specific enduracidin formation rate (B) during cultivation of *Streptomyces* sp. MC079 under different pH values for enduracidin production. pH–uncontrolled (curve 1), pH 5.5 (curve 2), pH 5.8 (curve 3), pH 6.0 (curve 4).

Table 2. Analysis of parameters under different fermentation culture conditions for enduracidin production by *Streptomyces* sp. MC079

Parameters	pH uncontrolled	pH 5.5	pH 5.8	pH 6.0	Two–stage pH control strategy	Constant–rate feeding strategy	pH shift feeding strategy
Culture time[a] (h)	160	160	160	160	152	160	152
Enduracidin concentration (mg/L)	35.42	40.23	46.21	31.12	53.57	44.43	31.23
Enduracidin productivity (mg/L/h)	0.369	0.419	0.525	0.324	0.609	0.462	0.697
Residual glucose (g/L)	0.1	0.08	0.06	0.31	0.06	0.08	0.05

[a]Fermentation time required to reached maximal enduracidin production.

but also increased enduracidin productivity. The results show that the two–stage pH control strategy remarkably improved the productivity of enduracidin by *Streptomyces* sp. MC079.

3.6. Effects of fed–batch fermentation by constant–rate feeding strategy on enduracidin production

To investigate the effects of glucose supplementation on our findings of enduracidin production, we added glucose to the final concentration of 40 g/L in the culture. Fed–batch culture is a preferred operational mode, which feeds substrate into a batch culture efficiently, and has the advantages of achieving a higher cell density by overcoming substrate inhibition and increasing production of the desired product [17]. To avoid potential delays or inhibition resulting from the sudden increases of glucose, a constant feeding approach was applied [18]. We used the protocol established by Li *et al*. [19] which a pH feedback-comtrolled substrate feeding strategy was built for high-level production of L-tryptophan by *Escherichia coli* in fed-batch culture.

Using the two–stage pH control strategy, glucose was consumed quickly, but the enduracidin production was not significantly increased. Therefore, fed–batch culture with a constant–rate feeding strategy was employed with an initial glucose concentration of 14 g/L. The initial broth volume for fed–batch culture was 3.0 L. When the residual glucose concentration was under 5 g/L, feeding of concentrated glucose (300 g/L) was started at the feeding rate of 6.25 mL/h by a computer–controlled pump, and the feeding volume was 300 mL. Fig. 6 shows the influence of the constant–rate feeding strategy on enduracidin production. The DCW reached a maximum value of 8.32 g/L at 80 h, suggesting that supplementation with glucose influences cell growth. The maximum concentration and productivity of enduracidin reached 44.43 mg/L and 0.462 mg/L/h in the constant–rate feeding fermentation process, which were both 12.5% higher than the results with pH uncontrolled batch fermentation (Table 2). However, the maximum DCW and enduracidin production were lower than the results of two–stage pH control strategy.

3.7. Effects of fed–batch fermentation by pH shift feeding strategy on enduracidin production

To compare a two–stage pH control strategy and constant–rate feeding strategy, a pH shift feeding strategy was employed with the same initial culture conditions and the constant–rate feeding strategy (see materials and methods section: 2.5 for details). When the pH naturally decreased from the initial pH of 7.4 ~ 5.5, the culture pH was maintained at pH 5.5 until 112 h of cultivation, and then

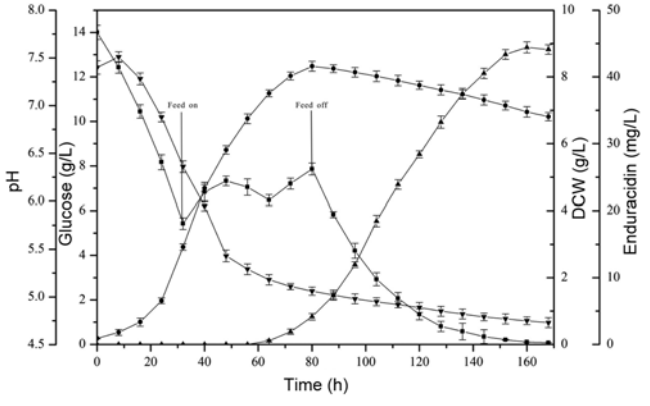

Fig. 6. Time profiles of cell growth, enduracidin concentration, glucose concentration and pH during fed-batch fermentation of *Streptomyces* sp. MC079 using constant–rate feeding strategy. Enduracidin (▲), DCW (●), pH (▼), Glucose (■).

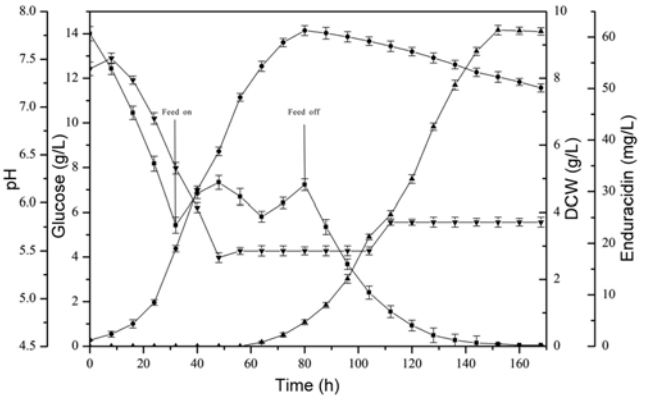

Fig. 7. Time profiles of cell growth, enduracidin concentration, glucose concentration and pH during fed-batch fermentation of *Streptomyces* sp. MC079 using pH shift feeding strategy. Enduracidin (▲), DCW (●), pH (▼), Glucose (■).

pH was shifted to 5.8 to further promote enduracidin formation in later fermentation. Fig. 7 shows the influence of the pH shift feeding strategy on enduracidin production, which glucose concentration was maintained at a low level under pH control strategy. Using this strategy, the DCW reached a maximum value of 9.43 g/L at 80 h. The maximum concentration and productivity of enduracidin reached 61.37 mg/L and 0.697 mg/L/h in the pH shift feeding strategy, which was 73.3 and 88.9% higher than the results with pH uncontrolled batch fermentation, respectively (Table 2).

4. Conclusion

In the present study, the effects of pH and glucose feeding strategies on enduracidin production were explored in order to identify the optimum strategy to maximize *Streptomyces* sp. MC079 growth and its subsequent enduracidin production. pH was identified as a key factor in enduracidin production by *Streptomyces* sp. MC079, the optimal range was 5.5-5.8. A two–stage pH control strategy was developed based on the kinetic analysis of efficient enduracidin fermentation using *Streptomyces* sp. MC079. By applying this pH control strategy, the maximum concentration of enduracidin reached 53.57 mg/L. In addition, a pH shift feeding strategy was used, and the maximum concentration and productivity of enduracidin reached 61.37 mg/L and 0.697 mg/L/h, which is 73.3 and 88.9% higher than the results with pH uncontrolled batch fermentation. Therefore, a pH shift feeding strategy of the fermentation process, as the one described in this study, could significantly increase the enduracidin production in *Streptomyces* sp. MC079 and can be applied to the industrial–scale production of enduracidin.

Acknowledgements

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References

1. Higashide, E., K. Hatano, M. Shibata, and K. Nakazawa (1968) Enduracidin, a new antibiotic. I. *Streptomyces fungicidicus* No. B5477, an enduracidin producing organism. *J. Antibiot. (Tokyo)* 21: 126-137.
2. Iwasaki, H., S. Horii, M. Asai, K. Mizuno, J. Ueyanagi, and A. Miyake (1973) Enduracidin, a new antibiotic VIII. Structures of enduracidins A and B. *Chem. Pharm. Bull*. 21: 1112-1191.
3. Zhou, M. J., Y. B.Yan, and J. Hu (2007) Progress in rearch of enduracidin. *Chin. J. Vet. Med.* 41: 42-44.
4. Miyake, A. (1972) Enduracidin derivatives. *US Patent* 3,694,548.
5. Matsumura, S. (1984) Production of enduracidin and microorganisms therefore. *US Patent* 4,465,771.
6. Xu, J. (2009) New enduracidin–producing strain and high–yielding mutant strain. *CN Patent* 200910253869.6.
7. Yin, X. and T. M. Zabriskie (2006) The enduracidin biosynthetic gene duster from *Streptomyces fungicidicus*. *Microbiol.* 152: 2969-2983.
8. Li, X. G., X. M. Tang, J. Xiao, G. H. Ma, L. Xu, S. J. Xie, M. J. Xu, X. Xiao, and J. Xu (2013) Harnessing the potential of halogenated natural product biosynthesis by mangrove–derived actinomycetes. *Mar. Drugs* 11: 3875-3890.
9. Xiao, J., J. Xu, S. J. Xie, X. Y. Zhang, Z. N. Yu, and J. Xu (2008) Isolation of mangrove actinomycetes and their antagonistic activities. *Chin. J. Appl. Environ. Biol.* 14: 244-248.
10. Ma, G. H. (2010) *A study of the enduracidin producing strain of Streptomyces atroviens and the biosynthetic gene cluster*. M.S. Thesis. Sun Yat–sen University, Guangzhou, China.
11. Vlaev, S., S. Rusinova–Videva, K. Pavlova, M. Kuncheva, I. Panchev, and S. Dobreva (2013) Submerged culture process for biomass and exopolysaccharide production by Antarctic yeast: Some engineering considerations. *Appl. Microbiol. Biotechnol.* 97: 5303-5313.
12. Ying, X. X., Z. Ping, L. Sha, C. X. Ye, Y. Zhong, and X. Hong (2014) Production of rhamsan gum using a two–stage pH control strategy by *Sphingomonas* sp. CGMCC 6833. *Appl. Biochem. Biotechnol.* 172: 168-175.
13. Luo, H. H., Y. Y. Niu, C. Q. Duan, H. J. Su, and G. L. Yan (2013) A pH control strategy for increased β–carotene production during batch fermentation by recombinant industrial wine yeast. *Proc. Biochem.* 48: 195-200.
14. Chen, X. S., S. Li, L. J. Liao, X. D. Ren, F. Li, L. Tang, J. H. Zhang, and Z. G. Mao (2011) Production of ε–poly–L–lysine using a novel two–stage pH control strategy by *Streptomyces* sp. M–Z18 from glycerol. *Bioproc. Biosyst. Eng.* 34: 561-567.
15. Hu, X. Q., J. Chu, S. L Zhang, Y. P. Zhuang, X. Wu, H. X Chen, Z. Y. Lv, and Z. Y. Yuan (2014) An alkaline pH control strategy for methionine adenosyltransferase production in *Pichia pastoris* fermentation. *Biotechnol. Bioproc. Eng.* 19: 900-907.
16. Zheng, M. X., M. Y. Zheng, Y. Y. Wu, H. L. Ma, and K. J. Wang (2015) Effect of pH on types of acidogenic fermentation of fruit and vegetable wastes. *Biotechnol. Bioproc. Eng.* 20: 298-303.
17. Li, Y., H. Jiang, X. Du, X. Huang, X. Zhang, and Y. Xu (2010) Enhancement of phenazine–1–carboxylic acid production using batch and fed–batch culture of gacA inactivated *Pseudomonas* sp. M18G. *Bioresour. Technol.* 101: 3649-3656.
18. Chen, X., L. Liu, J. Li, J. Liu, G. Du, and J. Chen (2012) Optimization of glucose feeding approaches for enhanced glucosamine and N–acetylglucosamine production by an engineered *Escherichia coli*. *J. Ind. Microbiol. Biotechnol.* 39: 359-365.
19. Cheng, L. K., J. Wang, Q. Y. Xu, C. G. Zhao, Z. Q. Shen, X. X. Xie, and N. Chen (2013) Strategy for pH control and pH feedback–controlled substrate feeding for high–level production of L–tryptophan by *Escherichia coli*. *World J. Microbiol. Biotechnol.* 29: 883-890.