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The pH Shift and Precursor Feeding Strategy in a Low-Toxicity FR-008/Candicidin Derivative CS103 Fermentation Bioprocess by a Mutant of *Streptomyces* sp. FR-008

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Abstract CS103, the novel derivative of polyene macrolides antibiotic FR-008/candicidin with lower toxicity has been isolated from the culture mycelia of the mutant of *Streptomyces* sp. FR-008, with targeted deletions of the *fscP* cytochrome P450 gene from its chromosome. To enhance biosynthesis of CS103, pH shift and precursor feeding strategy for fermentation process by the mutant of *Streptomyces* sp. FR-008 in a stirred tank bioreactor was developed. According to the process parameters analysis, the effectiveness of the strategy was examined and confirmed by experiments. A maximal CS103 concentration of 139.98 μg/mL was obtained, 2.05-fold higher than that in the pH-uncontrolled fermentation. Compared to other three cases as pH-uncontrolled, pH-controlled, and two-stage pH-controlled batch cultures, the proposed "pH shift and precursor feeding strategy" effectively avoided the scarcity of the antibiotic precursor, increased the CS103 yield from biomass ($Y_{P/X}$) and substrate ($Y_{P/S}$) by 110.61% and 48.52%, respectively, and at the time the fermentation time was shortened from 120 to 96 h. The highest CS103 production rate (1.46 μg mL^{-1} h^{-1}) of the pH shift and precursor feeding strategy was 284.21%, 97.30%, and 58.70% higher than that of pH-uncontrolled, pH-controlled, and two-stage pH-controlled batch culture cases, respectively.

Keywords Two-stage pH control strategy · Precursor feeding · Polyene macrolides antibiotic · Low-toxicity FR-008/candicidin · Fermentation

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Introduction

Polyene compounds constitute a large group of antibiotics harboring three to eight conjugated double bonds in the macrolactone ring. They usually form transmembrane channels by interacting with sterols in the eukaryotic cell membrane, causing leakage of small molecules and ions and consequent cell death [1]. Many polyene antibiotics, such as amphotericin, candicidin, nystatin, and pimaricin, are effective clinical antifungal agents; some of them also have antiviral, antibacterial, or immuno-stimulating activities [2]. Like other polyene antibiotics, candicidin also possess conjugated double bonds (seven), making it as potent antifungal agent mainly useful for the treatment of *Trichomonas vaginalis* infection. In addition, candicidin could kill mosquito larvae [3] and prevent benign prostatic hyperplasia [4]. Candicidin had been put in use for treating Moniliform colpitis, and it has a proven effect to cholesterol and bile acid metabolism [4, 5]. Structural studies revealed that candicidin contains an amino sugar in addition to the polyene macrolide lactone ring, mycosamine, and an aromatic moiety [6, 7], which is similar to the structure of amphotericin B. The FR-008 complex produced by *Streptomyces* sp. FR-008 is found to possess the identity to the candicidin complex (Fig. 1) [8] and holds a greater potential for pharmaceutical utility for its remarkably higher production yield than the originally discovered candicidin producer (*Streptomyces griseus* IMRU3570).

Polyene antibiotics have severe side effect to patients due to its affinity to sterol in human body, especially toxic to kidney. This limits their clinical utility, though they are still the choices for severe fungal affections. Although some carrier systems such as liposomes had been developed, the serious toxicity to human body still cannot be eliminated. Therefore, developing polyene derivatives with inherent reduced toxicity draws the attention of many scholars. Chemical modification has shown that suppression of charge on the exocyclic carboxyl group reduces toxicity and improves antifungal specificity [9]. In the study of amphotericin B, chemically synthesized decarboxyl amphotericin B has lower toxicity than amphotericin B. Modification of the exocyclic carboxyl group of amphotericin B is known to bring about a substantial reduction in its toxicity [9, 10]. When the polyene interacts with membrane sterols, the carboxyl group is contributed to an extensive network of hydrogen bonds that involves the mycosamine amino group, a water molecule, and the sterol hydroxyl group [10, 11]. This system of bonds is equally strong in polyene–cholesterol and polyene–ergosterol complexes. With a methyl ester, this network is weakened, so that the polyene binds more selectively to ergosterol through specific hydrophobic interactions within the lipid bilayer. FR-008/candicidin is chemically similar to amphotericin which do not harbor an aromatic side chain.

CS103, the novel decarboxylated FR-008/candicidin derivatives with lower toxicity has been isolated from the culture mycelia of the mutant of *Streptomyces* sp. FR-008, with targeted deletions of the cytochrome P450 gene (*fscP*) from its chromosome (Fig. 1; Chen

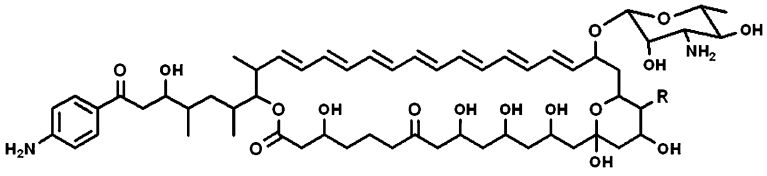

Fig. 1 Chemical structures of FR-008/candicidin and the novel decarboxylated derivatives CS103 R=COOH is FR-008/candicidin; R=CH3 is its novel decarboxylated derivatives CS103

and Mao et al., unpublished). The toxicity of decarboxylated FR-008/candicidin derivatives measured as hemolytic activity has been shown to be greatly reduced compared to wild-type product, which is about 50 times lower than FR-008/candicidin (Chen and Mao et al., unpublished). Nevertheless, the yield of these chemicals is quite low, which makes deeply clinical study, and industrialization comes across an obstacle. Mao et al. had optimized the production medium using statistical experimental method, which is effective on FR-008/candicidin and a series of their novel polyenes derivatives [12].

The pH is one of the most important environment parameters affecting cell growth and product formation. Previous studies had demonstrated that pH played a central role on the microbe growth and metabolite accumulation [13–15]. In general, the effects of pH on the cell growth and product accumulation vary with the different microorganisms, medium composition, and operation conditions. To our knowledge, there have been few reports about the effects of pH on the production of antibiotic FR-008/candicidin (including their derivatives), biosynthesis of their precursor, and cell growth in batch fermentation in the stirred tank bioreactor. The aim of this work was to evaluate the effects of different pH-controlled strategies on antibiotic formation, precursor biosynthesis, and cell growth in batch fermentation by the *Streptomyces* sp. FR-008 mutant. Subsequently, a pH shift and precursor feeding strategy was proposed to enhance the accumulation of the low-toxicity FR-008/candicidin derivative CS103 based on the kinetic analysis of batch processes in a 3.7-L stirred fermentor. The effectiveness of the improved strategy was verified experimentally. Therefore, results and findings of the current study provided a solid basis for the industrialization of FR-008/candicidin and its novel derivatives and made these polyene antibiotics more cost-effective.

Materials and Methods

Materials

Tryptone soya broth, malt extract, yeast extract, and peptone were obtained from Oxiod Ltd. All other reagents used were of analytical grade.

Microorganism

Streptomyces sp. FR-008 and its mutants were used in this study. CS103, the mutant of *Streptomyces* sp. FR-008, with targeted deletions of the cytochrome P450 gene (*fscP*) from its chromosome, was constructed as follows: pJTU28 is a pIJ2925 derivative with a 2.3-kb *Pst*I–*Kpn*I fragment carrying *fscP* which is derived from a re-ligation of *Pst*I-digested pJTU26. After *Bss*HII digestion, a 396-bp sequence inside *fscP* gene was removed, followed by an insertion of a 1.4-kb *Bss*HII fragment from pJTU33 carrying the apramycin resistance gene [*acc(3)IV*]to gain pJTU37. The 3.7-kb *Bgl*II fragment including inserted apramycin resistance gene was recovered from pJTU37 for ligation into the *Bam*HI site of pHZ1358 to generate pJTU50 for targeted replacement of *fscP* gene. pJTU50 was transferred into *Streptomyces* sp. FR-008 by conjugation from *Escherichia coli* ET12567 carrying RP4 derivative pUZ8002 [8]. Thiostrepton-sensitive and apramycin resistance ($Thio^S$-Apr^R) colonies were counter-selected from the initial $Thio^R$ exconjugants after two rounds of nonselective growth to obtain CS103 (Chen and Mao et al., unpublished). Spore suspensions were prepared by scraping off spores with a plastic spatula from sporulation medium and stored as 1 mL aliquots in 20% glycerol at −72°C.

Medium and Growth Conditions

The seed culture was grown in a 250-mL flask containing 50 mL medium composed of glucose 10 g/L, malt extract 3 g/L, yeast extract 3 g/L, and peptone 5 g/L on a rotary shaker at 200 rpm and 28°C for 30 h. Then, the seeds are inoculated to the bioreactors filled with fermentation medium. The production medium was: glucose 20 g/L, glycerol 13.89 mL/L, soluble starch 10 g/L, yeast extract 3 g/L, peptone 1.01 g/L, industrial complex aminophenol 5 g/L, ferrous sulfate 0.5 g/L, copper sulfate 0.0428 g/L, and sodium chloride 10 g/L [12]. A 3.7-L bioreactor (KLF2000 3.7 L, Bioengineering AG, Switzerland) equipped with a pH electrode, a dissolved oxygen electrode, and two peristaltic pumps was used to scale up. The fermentor was inoculated with 10% (*v*/*v*) pre-culture and operated at 28°C. The pH value was adjusted with NaOH and HCl and maintained automatically through the cultures. The aeration rate was 200 L/h, with speeds of 450 rpm used for agitation. All experiments were repeated thrice.

Analytical Method

The novel FR-008/candicidin derivatives were determined by high-performance liquid chromatography. A SB-C8 column ZORBAX (4.6×250 mm, 5 μm, Agilent Technology Co.) was used. The mobile phase consisted of acetonitrile–acetic ammonium (20 mmol/L, pH 4.0; 40:60, *v*/*v*), the flow rate was 1.0 mL/min, the UV detection wavelength was 380 nm, and the whole process was performed at 25°C [16].

The mycelial biomass was evaluated by weighing the dry cell amount. Samples collected at different times were centrifuged at 10,000 rpm for 10 min, and the resulting precipitate was washed repeatedly with distilled water and dried at 80°C until a constant weight was achieved to get the dry cell weight (DCW) measurement. Residual glucose in the fermentation broth was determined by the glucose kit (Shanghai Institute of Biological Products, China). The nitrogen concentration was determined by the formaldehyde method. The pH was measured by the pH electrode and adjusted with NaOH and HCl. The dissolved oxygen concentration (DO) was monitored with an online probe. The oxygen and CO_2 concentrations in the off-gas were measured by a gas analyzer (Ultramat 23, Siemens, Munich, Germany) and used to calculate the respiratory quotient (RQ), which was defined as the molar ratio of CO_2 produced to O_2 consumed.

Results and Discussion

Effect of pH-Uncontrolled and pH-Controlled on Fermentation Course

According to the research in shake flasks, we found that the optimum pH value for antibiotic CS103 synthesized was maintained at pH 6.5–7.5. The fermentation of antibiotic CS103 by the *Streptomyces* sp. FR-008 mutant was performed at pH-uncontrolled case (the initial pH at about 7.0, pH was not controlled in the fermentation process) in a 3.7-L stirred fermentor; the results are presented in Fig. 2. In the pH-uncontrolled fermentation process, the pH increased to 7.5 at the early period (0–12 h) and then dropped to 5.5 during 12–96 h. These results indicated that the pH variation could be regarded as a metabolism level signal of the microorganism and its intermediate metabolites. What is more is that it is important to investigate the effect of pH on the mycelial growth, primary metabolites, and secondary metabolites at different fermentation phase to enhance the antibiotic CS103 production.

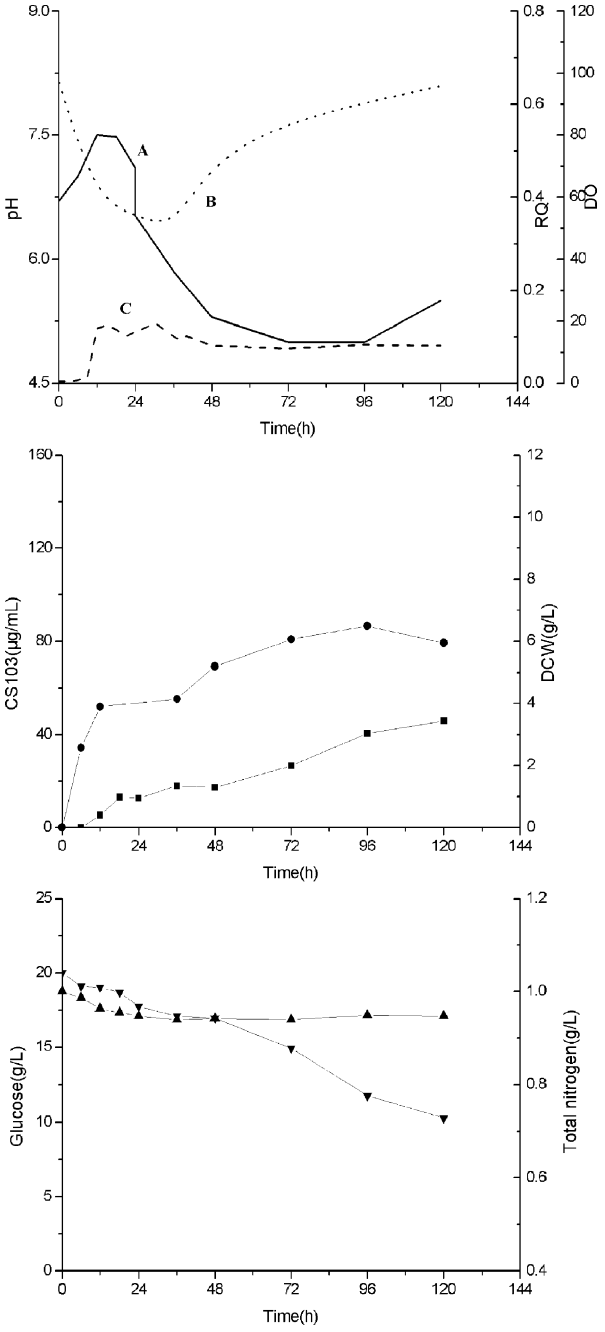

Fig. 2 The time course of the fermentation process with natural pH on the production of CS103. **a** pH, **b** DO, **c** RQ. *Square*, production of CS103; *circle*, DCW; *inverted triangle*, concentration of glucose; *triangle*, concentration of nitrogen source

The glucose consumed as the biomass increased, and the secondary metabolic production was strongly associated with mycelial growth. The glucose and its intermediate metabolite were not only the energy source for cell growth but also important precursors of FR-008/candicidin. The importance of pH-controlled strategy on fermentation process of

antibiotic CS103 is illustrated in Fig. 3 and Table 1 between the pH-controlled process and pH-uncontrolled process. In the two fermentation process, the antibiotic CS103 synthesis initiated at about 12 h and rapidly increased after 48 h. The maximum production of CS103 (P_{max}) increased from 45.87 μg/mL of the uncontrolled process to 88.24 μg/mL of the

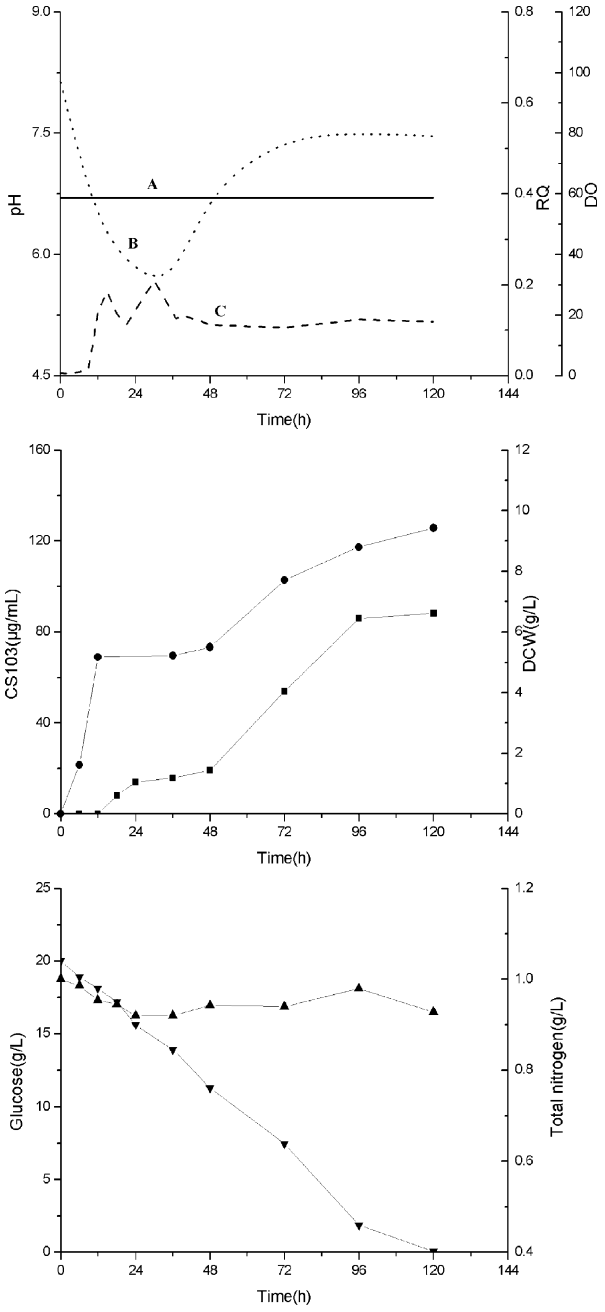

Fig. 3 The time course of the fermentation process with pH6.9 on the production of CS103. **a** pH, **b** DO, **c** RQ. *Square*, production of CS103; *circle*, DCW; *inverted triangle*, concentration of glucose; *triangle*, concentration of nitrogen source

Table 1 Detail data of the four fermentations.

Experiment	DCW (g/L)		P_{max} (μg/mL)		$Y_{P/X}$[a] (μg/g)		$Y_{P/S}$[b] (μg/g)		Production rate[c] (μg mL^{-1} h^{-1})		Glucose consumed (g)
	Values	Increased (%)	Values	Increased (%)	Values	Increased (%)	Values	Increased (%)	Values	Increased %)	
pH-Uncontrolled	6.49	–	45.87	–	7.07	–	4.72	–	0.38	–	9.72
pH-Controlled	9.43	45.30	88.24	92.37	9.36	32.39	4.42	−6.36	0.74	94.74	19.94
Two-stage pH-controlled	9.52	46.69	110.74	141.42	11.63	64.50	5.54	17.37	0.92	142.11	19.99
pH Shift and precursor feeding	9.4	44.84	139.98	205.17	14.89	110.61	7.01	48.52	1.46[d]	284.21	19.97

[a] Yield of product from biomass (μg of product per g of biomass)

[b] Yield of product from substrate (μg of product per g of glucose)

[c] Production rate represents volumetric CS103 production rate (μg mL^{-1} h^{-1})

[d] The P_{max} obtained with pH shift and precursor feeding strategy is at 96 h, others is at 120

controlled process (increased by 92.37%), and the biomass content of controlled process was 9.43 g/L, 45.30% higher than that in pH-uncontrolled case (6.49 g/L). Therefore, the yield of product from biomass ($Y_{P/X}$) was enhanced from 7.07 μg/g of the uncontrolled process to 9.36 μg/g of the controlled process (increased by 32.39%).

The concentration of glucose and nitrogen source in culture broth were also detected. The results showed that when the fermentation was performed at natural pH, the consumption rate of the glucose and nitrogen source was slower than that of the pH-controlled process. In the pH-controlled process, glucose utilization proceeds linearly until it is almost depleted, while in the case of pH-uncontrolled, the utilization rate slows down due to the lower growth rate.

As shown in the figures (Figs. 2 and 3), we could see that cell growth was associated with the increase of RQ, which results in a decrease in the DO concentration of the culture. When the antibiotic biosynthesis started, there was a sharp increase in DO and a decrease in RQ. In the pH-controlled process, the metabolism of the strain got faster, the DO concentration was lower, while the RQ was higher, respectively, than those in the case of pH-uncontrolled.

Effect of pH Shift Strategy on Biosynthesis of Antibiotic CS103

The above results indicated that the pH played a vital role in microorganism metabolism, antibiotic formation, and cell growth. Gil et al. [2] found that the activity of *p*-aminobenzoic acid synthase was affected by the fermentation pH remarkably (pH 9.0 is the most suitable pH value), and it could convert chorismic acid to *p*-aminobenzoic acid at the beginning of the fermentation, which was a significant precursor of the aromatic *p*-aminoacetophenone moiety of FR-008/candicidin and their derivatives such as CS103. But in our former study, we found that in the secondary metabolite pathway, the optimum fermentation pH for antibiotic CS103 synthesized was 7.0. We developed the two-stage pH controlling strategy for CS103 production based on the above results.

The batch cultures (BC) were designed as follows: BC_A, the culture pH was controlled at pH 6.90 in the whole process; BC_B, the culture pH was controlled at pH 7.50 around 0–20 h and then shifted to pH 6.90 in the secondary stage; BC_C, the culture pH was controlled at pH 8.0 around 0–20 h and then shifted to pH 6.90 in the secondary stage; BC_D, the culture pH was controlled at pH 8.50 around 0–20 h and then shifted to pH 6.90 in the secondary stage; BC_E, the culture pH was controlled at pH 9.0 around 0–20 h and then shifted to pH 6.90 in the secondary stage. The microorganism was cultured on rotary shakers at 200 rpm and 28°C for 120 h. The time courses of CS103 concentration at different pH-controlled strategies are shown in Fig. 4.

It was shown that operation BC_C could enhance the antibiotic accumulation and the culture pH in the beginning of the fermentation was pH 8.0. Then, we studied the shift points of pH values. The culture pH was controlled at pH 8.0 at the beginning of the culture and then adjusted to pH 6.90 after 6, 12, 18, and 24 h, respectively. The microorganism was cultured in rotary shakers at 200 rpm and 28°C for 120 h. Results indicated that the production of CS103 was dramatically improved when the pH value was shifted to 6.90 at 24 h after inoculation (data not shown). The above results demonstrated that a higher pH at early fermentation stage (0–24 h) was demonstrated to be advantageous to the synthesis of the precursor of antibiotic CS103, while a proper increase in pH of the submerged culture at mid- and later stage could increase antibiotic CS103 production. Then, we developed an optimal pH shift strategy for FR-008/candicidin and their derivative CS103 production as follows: The fermentation pH was kept at 8.0 during the first 24 h and then switched to

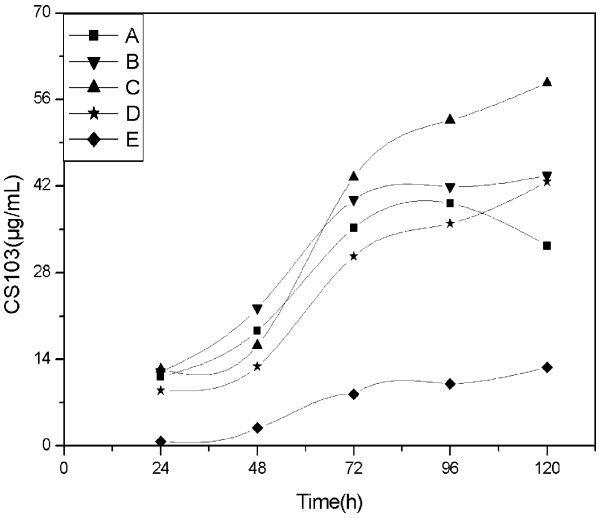

Fig. 4 Influence of different pH controlling strategy on the production of CS103 in flasks. **a** Culture pH was controlled at pH 6.90 in the whole process (*square*). **b** Culture pH was controlled at pH 7.50 around 0–20 h and then shifted to pH 6.90 in the secondary stage (*inverted triangle*). **c** Culture pH was controlled at pH 8.0 around 0–20 h and then shifted to pH 6.90 in the secondary stage (*triangle*). **d** Culture pH was controlled at pH 8.50 around 0–20 h and then shifted to pH 6.90 in the secondary stage (*star*). **e** Culture pH was controlled at pH 9.0 around 0–20 h and then shifted to pH 6.90 in the secondary stage (*diamond*)

6.9 at 24 h after inoculation. The time courses of CS103 fermentation are shown in Fig. 5 and the results of pH shift strategy and the different pH experiments are summarized in Table 1. With the two-stage control strategy, the maximum production of CS103 (P_{max}) increased to 110.74 μg/mL, higher than that of pH-uncontrolled (increased by 141.42%) and pH-controlled at 6.90 (increased by 25.5%) cases. The maximum DCW (9.52 g/L) was increased by 45.30% from uncontrolled process (6.49 g/L), while not significantly improved from pH-controlled batch culture (9.43 g/L). As expected, the $Y_{P/X}$ (yield of product from biomass) and $Y_{P/S}$ (yield of product from substrate) of pH-shift-controlled fermentation process reached 11.63 and 5.54 μg/g, respectively, enhanced by 64.5% and 17.37%, respectively, as compared to that of the pH-uncontrolled fermentation case.

In the fermentation of pH shift strategy, glucose and nitrogen source utilization rates were higher than in the case of constant pH controlled at pH 6.90 (Figs. 4 and 5). Especially, the glucose was consumed completely at 96 h in the pH shift strategy while at 120 h in the former case. What is more is that for the enhancement of the metabolism, the maximum RQ increased to 0.33.

pH Shift and Precursor Feeding Strategy Enhanced the Biosynthesis of Antibiotic CS103

p-Aminobenzoic acid (PABA) is a significant precursor of FR-008/candicidin and their derivatives such as CS103. To investigate the effects of adding PABA on the synthesis of antibiotic CS103, it was added at the doses of 0.02, 0.04, 0.06, 0.08, and 0.10 g/L. We found that the addition of PABA at a concentration of 0.02 g/L increased the synthesis of antibiotic CS103 as compared to that of the control. On the contrary, the addition of PABA at the high concentration (0.10 g/L) inhibited the synthesis of CS103. The effect of precursor feeding mode on the yields of CS103 was investigated in rotary shakers. Pulse feeding experiments were designed as follows, one-pulse feeding, two-pulse feeding, and four-pulse feeding experiments. The results showed that when the same amount of PABA was fed at 0, 12, 24, and 48 h (four-pulse feeding), the yield of antibiotic CS103 obtained was 33.03% and 14.40% higher than that of one-pulse feeding and two-pulse feeding experiments, respectively. This higher strength of PABA stimulation by repeated fed-batch

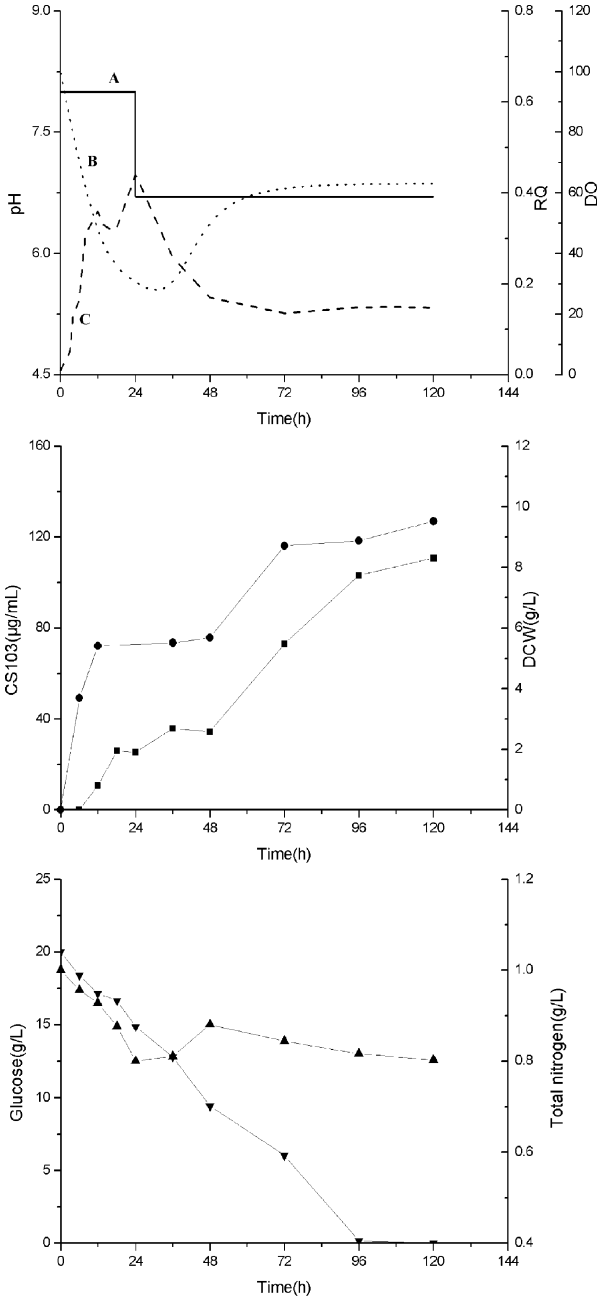

Fig. 5 The time course of the fermentation process with two-stage pH controlling strategy on the production of CS103. **a** pH, **b** DO, **c** RQ. *Square*, production of CS103; *circle*, DCW; *inverted triangle*, concentration of glucose; *triangle*, concentration of nitrogen source

cultivation suggested that smooth modification of physiological environments (e.g., concentration of PABA) might have a positive role on the biosynthesis of antibiotic CS103.

According to the study of the precursor feeding strategy for the production of secondary metabolites, with the pH shift strategy, 0.02 g/L PABA was fed to the 3.7-L stirred bioreactor at 0, 12, 24, and 48 h. The results are shown in Fig. 6 and Table 1. When

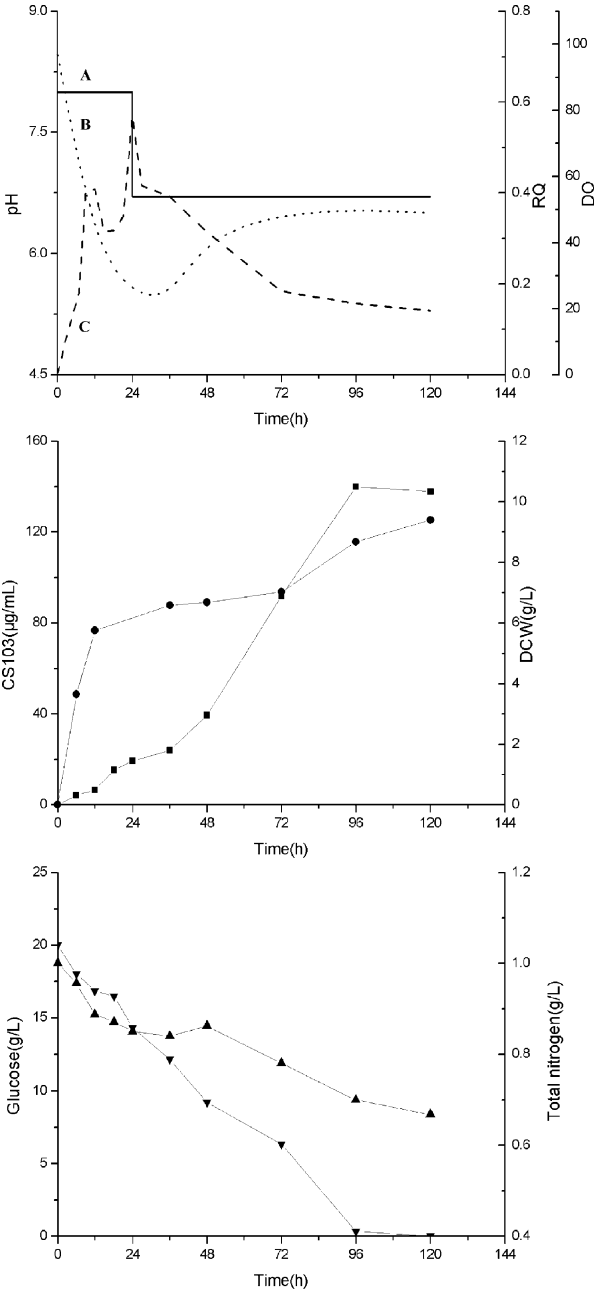

Fig. 6 The time course of the fermentation process with pH-shift and precursor feeding strategy on the production of CS103. **a** pH, **b** DO, **c** RQ. *Square*, production of CS103; *circle*, DCW; *inverted triangle*, concentration of glucose; *triangle*, concentration of nitrogen source

preformed according to the pH shift and precursor feeding strategy, the maximum antibiotic concentration reached 139.98 μg/mL at 96 h (other batch cultures was at 120 h), which was increased 205.17%, compared with that of pH-controlled batch culture. Comparing with the other three cases (pH-uncontrolled, pH-controlled, and two-stage pH controlled batch cultures), the fermentation time was shortened from 120 to 96 h. The highest CS103

production rate (1.46 μg mL^{-1} h^{-1}) of using the pH shift and precursor feeding strategy was 284.21%, 97.30%, and 58.70% higher than that of pH-uncontrolled, pH-controlled, and two-stage pH-controlled batch culture cases, respectively. On the other hand, with the pH shift and precursor feeding strategy, the maximum DCW (9.4 g/L) of the microorganism was not obviously influenced compared with the pH-controlled (9.43 g/L) and two-stage pH-controlled (9.52 g/L) cases. These results demonstrated that the proposed pH shift and precursor feeding strategy could efficiently enhance the antibiotic biosynthesis as well as improve the $Y_{P/X}$ (yield of product from biomass), $Y_{P/S}$ (yield of product from substrate), and glucose consumption (Table 1) in the fermentation process. The production of polyene antibiotic started at a time when glucose utilization was maximal in the four different cases. For the sharp increase in DO and decrease in RQ associated with the biosynthesis phase of CS103, the maximum RQ at the turning point of the metabolism increased to 0.56, suggesting that in the four culture strategies, the pH shift and precursor feeding mode was the best strategy to enhance the metabolism of the *Streptomyces* sp. FR-008 mutant.

Conclusion

Because of the biological activities and effect to cholesterol and bile acid metabolism, the antibiotic CS103 could be applied to some potential medicine. It is necessary to improve CS103 production through optimization of fermentation parameters. Since the production of CS103 by the *Streptomyces* sp. FR-008 mutant was highly influenced by the pH profile employed during fermentation, an improved two-stage pH control strategy for the low-toxicity FR-008/candicidin derivative CS103 fermentation was developed based on the analysis of the batch cultivation. According to the kinetic parameters analysis, the pH shift strategy, which controlled the fermentation pH at 8.0 in the first 24 h and then switched to pH 6.90, was successfully verified. Higher concentration (110.74 μg/mL) and productivity (0.92 μg mL^{-1} h^{-1}) of CS103 were achieved by applying this strategy. While in the culture with non-pH control, the antibiotic production and the productivity are 45.87 μg/mL and 0.38 μg mL^{-1} h^{-1}, respectively. Then, we investigated the precursor feeding strategy in the fermentation process. A maximal CS103 concentration of 139.98 μg/mL was obtained by feeding 0.02 g/L *p*-aminobenzoic acid at 0, 12, 24, and 48 h, which was 2.05-fold higher than that in the control fermentation. The highest CS103 production rate (1.46 μg mL^{-1} h^{-1}) obtained using the pH shift and precursor feeding strategy was 284.21%, 97.30%, and 58.70% higher than that of pH-uncontrolled, pH-controlled, and two-stage pH-controlled batch culture cases, respectively. The proposed strategy effectively avoided the scarcity of the antibiotic precursor, and it increased the yield of product from biomass ($Y_{P/X}$) and substrate ($Y_{P/S}$) by 110.61% and 48.52% compared to those of the batch culture with pH-uncontrolled case, respectively.

In this study, we also investigated the relationship between DO, RQ, cell growth, and the accumulation of CS103. As shown in the figures, cell growth was associated with the increase of RQ, which results in a decrease in the DO concentration of the culture. Roszkowski et al. found that since the NADP/NADPH ratio is maintained during macrolide biosynthesis [17], NADP reduction requires a certain activity of the NADPH-regenerating enzymes associated with the primary metabolism pathways. At about 24 h after inoculation, antibiotic synthesis was obviously initiated, thus requiring large amounts of NADPH for CS103 biosynthesis. Large changes in the level of DO should occur in the transition from primary to secondary metabolism. The sharp increase in DO and decrease in RQ associated

with the biosynthesis phase of CS103 suggested that a rapid change in the type of metabolism occurs at the turning point (Figs. 2, 3, 5 and 6).

These results demonstrated that the production of antibiotic CS103 was sensitive to the pH change in submerged culture, which resulted in the accumulation of the precursor for the antibiotic synthesis. The "pH shift and precursor feeding strategy" in the fermentation process of the aroma polyene macrolide antibiotics was reported for the first time. This work will lay a solid theoretic foundation for the fermentation scale-up process of the low-toxicity FR-008/candicidin derivatives.

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