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ORIGINAL RESEARCH PAPER

Improved production of pristinamycin coupled with an adsorbent resin in fermentation by *Streptomyces pristinaespiralis*

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Abstract Batch fermentation by *Streptomyces pristinaespiralis* with the addition of adsorbent resins was used to increase the production of pristinamycin. In consideration of the adsorption capacity and the desorption ability, a polymeric resin, JD-1, was finally selected. The maximum production of pristinamycin in Erlenmeyer flasks went up to 1.13 from 0.4 g l^{-1}, by adding 12% (w/v) resin JD-1 into the culture broth at 20 h after inoculation. In a 3 l bioreactor, pristinamycin fermentation with the addition of 12% (w/v) resin JD-1 at 20 h after inoculation reached 0.8 g l^{-1}, which was a 1.25-fold increase over fermentation without resin.

Keywords Adsorbent resin · Fermentation · Pristinamycin · Separation · *Streptomyces pristinaespiralis*

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Introduction

Pristinamycin, a member of the streptogramin family of antibiotics, consists of two different groups of ring-like structures (Preud'Homme et al. 1986). It has strong activity against methicillin-, penicillin-, and vancomycin-resistant bacteria, and exhibits a prolonged post-antibiotic effect (Qadri et al. 1997; Abdel-Hamid and Phillips 2003). Pristinamycin is produced extracellularly by *Streptomyces pristinaespiralis* (Paquet et al. 1992; Corvini et al. 2000, 2004; Voelker and Altaba 2001) but, during fermentation, it is enzymatically, degraded. Moreover, both the growth of *S. pristinaespiralis* and the synthesis of pristinamycin are inhibited by pristinamycin itself. The reported highest yield is just over 100 mg l^{-1} due to these problems. Although much attention has been paid to genetic manipulations for pristinamycins production (Blanc et al. 1995, 1997; Hopwood 1997; Bamas-Jacques et al. 1999), the yield cannot reach a satisfactory level even by engineered strain without a favorable external condition. For the above reasons, removing the antibiotic simultaneously during the fermentation could be a solution. Recently, an integrated fermentation–separation system has been successfully developed to improve the fermentative productivity and process efficiency. Among in situ separation techniques, solid adsorbents have

been used to improve the production and recovery of the desired product (Marshall et al. 1990; Kim et al. 1999; Yu et al. 2002; Wang et al. 2004). For example, adding Diaion HP-20 into a fermentation led to a 4.2-fold increase in the production of teicoplanin (Lee et al. 2003).

In this paper, the adsorption technique was adopted to improve the production of pristinamycin in fermentation by *S. pristinaespiralis*. Batch fermentation of pristinamycin added with an adsorbent resin in a bioreactor was compared with the conventional fermentation without an adsorbent resin.

Materials and methods

Microorganism and medium

Streptomyces pristinaespiralis XC 505 was isolated by mutation with UV and chemical mutagens from a wild-type strain. It was grown on a medium consisting of (g l^{-1}): soluble starch, 15; glucose, 10; soybean flour, 15; peptone, 5; yeast extract, 5; KNO_3, 2.5; NaCl, 2; $CaCO_3$, 4; and the pH value was adjusted to 7.1 ± 0.1. Production medium consisted of (g l^{-1}): soluble starch, 40; glucose, 10; soybean flour, 25; peptone, 5; yeast extract, 3; fish extract, 10; $(NH_4)_2SO_4$, 1.5; $MgSO_4 \cdot 7H_2O$, 3.5; KH_2PO_4, 0.2; $CaCO_3$, 4; and the pH value was adjusted to 6.5 ± 0.1. All these media were sterilized for 20 min at 118°C.

Adsorbent resins

Resins, HP-20 (Mitsubishi, Tokyo, Japan), XAD-16 (Rohm and Hass, Philadelphia, PA, USA), JD-1 (Jianyang Pharmaceutical Co. Ltd, P.R. China) and HZ-817 (Huachang Polymer Co. Ltd, Shanghai, P.R. China) were soaked and swollen in acetone for 24 h to remove impurities. The acetone was then removed by washing with sufficient distilled water. The prepared resin was added to the medium before sterilization, or sterilized separately at 121°C for 20 min and then added to the culture broth at the designated time.

Fermentation with absorbent resins

The seed culture was inoculated at 6% (v/v) into Erlenmeyer flasks containing the production medium and the given content of adsorbent resins. The fermentation was carried out at 28°C with shaking at 220rpm for 72 h. For batch cultures in a bioreactor, 90 ml seed culture was added into a KF-3l fermentor (KBT, Korea) with 1.5 l production medium at 28°C and an aeration rate of 1 vvm. The dissolved O_2 level was maintained at a minimum of 30% air saturation and controlled by stirring conditions.

Analytical procedures

To determine the concentration of pristinamycin, one volume of culture sample containing both mycelia and resins was directly mixed with two volumes of acetone for 60 min, to extract the product from the resins and mycelia. Subsequently, the mixture was centrifuged at 2000*g* for 10 min, and then filtered with a 0.45 μm filter to gain the supernate. Finally, the pristinamycin was determined by HPLC with a 4.6 mm × 250 mm Hypersil ODS C_{18} column. For HPLC determination, the acetonitrile/water (45:55, v/v) was used as the mobile phase at 1.2 ml min^{-1} and the eluate was monitored at 206 nm. Commercial Pyostacine from Rhone-Poulenc Rorer Co. (Montrouge, France) was used as a reference standard.

For determining the dried cell weight (DCW), the culture sample was passed through a 0.3 mm stainless steel sieve to separate the resins, and then centrifuged at 2500× *g* for 15 min. The mycelia were therefore obtained and dried to constant. The supernate was simultaneously analyzed for the amino nitrogen by the formaldehyde titration method.

Results

Toxic effects of pristinamycin

The growth of *S. pristinaespiralis* was inhibited by pristinamycin in a dose-dependent manner with almost completely inhibition above 0.48 mg ml^{-1} (Fig. 1).

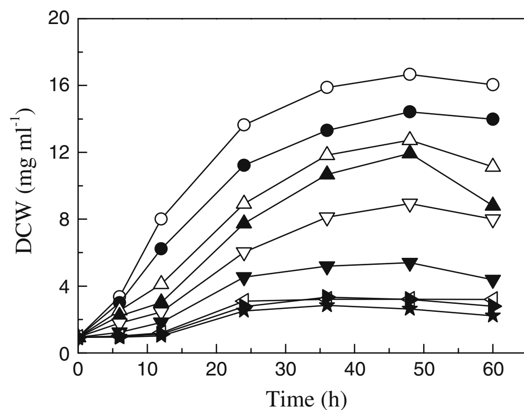

Fig. 1 Toxic effect of pristinamycin on the growth of *S. pristinaespiralis*. Pristinamycin was externally added into the medium at the inoculation stage with the concentration of (○) 0 mg ml^{-1}, (●) 0.02 mg ml^{-1}, (Δ) 0.04 mg ml^{-1}, (▲) 0.08 mg ml^{-1}, (∇) 0.16 mg ml^{-1}, (▼) 0.32 mg ml^{-1}, (◀) 0.48 mg ml^{-1}, (▶) 0.64 mg ml^{-1}, and (★) 0.80 mg ml^{-1}

Selection of adsorbents

The resins, HP-20, XAD-16, JD-1, and HZ-817, were tested in 0.5 mg ml^{-1} pristinamycin solution (Table 1). Resin XAD-16 had the highest capacity of adsorption. Desorption of the antibiotic from the resins was highest with JD-1 and HP-20. The best performance of adsorption and desorption was by JD-1, which was used for further study.

Effect of adsorbent content on pristinamycin production

The growth of *S. pristinaespiralis* gradually decreased with the increase of the content of added JD-1 resin, while the production of pristinamycin reached the maximum of 1.13 g l^{-1} when 12% (w/v) JD-1 was added (Fig. 2). This maybe attributed to the transfer of the antibiotic from the broth into the adsorbent, thus the end-product regulation and the biodegradation are avoided to some extent.

Table 1 Adsorption capacity and desorption ability of resins on pristinamycin

Resin	Adsorption capacity (mg g^{-1})[a]	Desorption ratio (%)[b]
HP-20	18.7 ± 1.0	80.2 ± 0.9
XAD-16	29.5 ± 0.9	73.9 ± 1.1
JD-1	25.7 ± 1	84.2 ± 1
HZ-817	18.7 ± 1.1	75.4 ± 1.3

[a]Resin, 0.2 g, was added into pristinamycin at of 0.5 mg ml^{-1}. The adsorption capacity was calculated from the initial and the residual concentrations of pristinamycin in solutions. The data are given as the mean of three replicates ± the standard deviation

[b]The value was calculated from the amounts of pristinamycin in the acetone eluent and bound by resin

Effect of addition time of adsorbent on pristinamycin production

Figure 3 indicates that the DCW had gradually increased from 70 to 84% of the control case when the JD-1 was added from 0 to 20 h after the inoculation, and the pristinamycin production had a corresponding increase of 1.83-fold when adding the JD-1 at 20 h of cultivation. Because the onset of pristinamycin synthesis was at about 20 h (shown in Fig. 4d), the addition of JD-1 after 20 h led to the decrease of both the pristinamycin production and the DCW, which arose from the toxic effect and feedback regulation of the antibiotic itself.

Batch fermentation of pristinamycin in a 3 l bioreactor

The batch fermentation of pristinamycin in a 3 l bioreactor was carried out with and without the JD-1, and the results are shown in Fig. 4. The growth inhibition due to the resin addition (Fig. 4a) was similar to the results from the Erlenmeyer flasks (see Figs. 2 and 3). Further, the growth ceased and the mycelia gradually autolyzed after 38–42 h of cultivation resulting in the increase of ammonia nitrogen (Fig. 4c). On the other hand, the addition of the resin brought a significant variation of the pH value, compared with a less change in the conventional fermentation (Fig. 4b). In the case of the JD-1 addition, the production of pristinamycin reached the maximum value of 0.8 g l^{-1} at 50 h of cultivation (Fig. 4d). But in the case without the resin, the production only reached 0.36 g l^{-1} at 42 h of cultivation and after that it gradually decreased. A possible reason for the difference between the two cases could be that the feedback regulation and the biodegradation had been avoided due to the binding of the pristinamycin to the added resin.

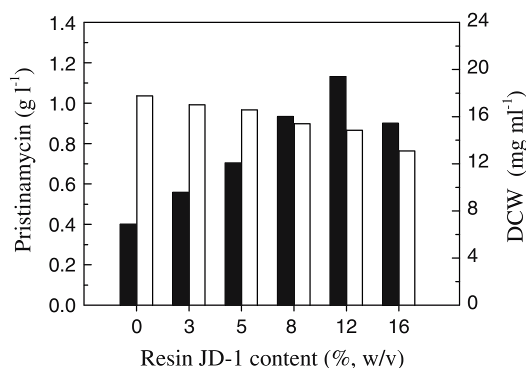

Fig. 2 Effect of resin JD-1 content on (□) mycelium growth and (■) pristinamycin production. Sterilized JD-1 at given amount was manually added into the culture broth at 20 h after inoculation

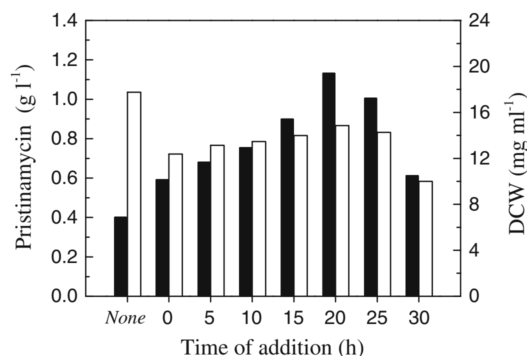

Fig. 3 Effect of addition time of JD-1 on (□) mycelium growth and (■) pristinamycin production. Twelve percent (w/v) of the sterilized resin JD-1 was manually added into the culture broth at the designated time after inoculation. None represents the control without resin

Discussion

Pristinamycin fermentation with the addition of resin JD-1 increased the production from 0.36 to 0.8 g l^{-1}, and therefore simplified the recovery procedure. Because the pristinamycin inhibits mycelial growth and its own production, the addition of the macroreticular resin JD-1 can eliminate the growth inhibition and feedback regulation to some extent, which provides a favorable environment for the biosynthesis of pristinamycin. From the practical point of view, the work laid a basis for further study on the

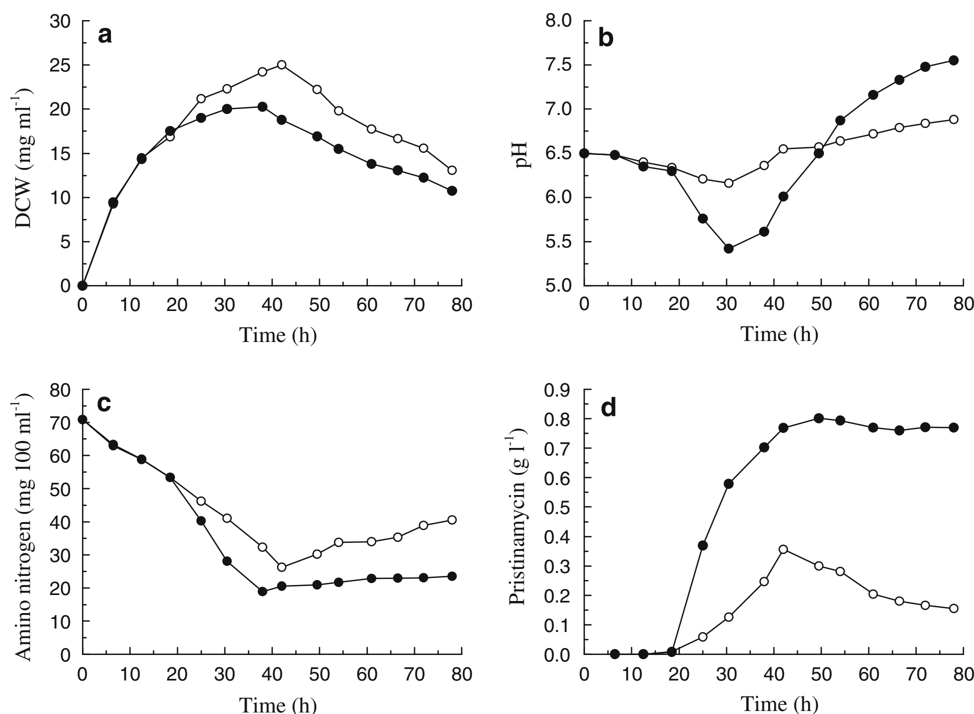

Fig. 4 Time courses of mycelium growth, pH variation, amino nitrogen consumption, and pristinamycin production by S. pristinaespiralis in a 3 l bioreactor. **(a)** DCW (mg ml^{-1}); **(b)** pH; **(c)** amino nitrogen (mg 100 ml^{-1}); **(d)** pristinamycin (g l^{-1}) [●, with the addition of 12% (w/v) JD-1 at 20 h after inoculation; ○, without the JD-1]

application of the in situ product removal technique to the pristinamycin production. At present, developing this kind of integrated bioprocess for pristinamycin production is under the way.

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