MICROBIAL CONVERSION OF ANTHRACYCLINE ANTIBIOTICS IV. STUDY ON THE GLYCOSIDATION OF ε-PYRROMYCINONE BY *STREPTOMYCES GALILAEUS* OBB-111-848

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In the previous paper[1], we reported microbial glycosidation of various anthracycline aglycones by a pigment-negative mutant OBB-111-848 which was derived from *Streptomyces galilaeus* OBB-111, an auramycins[2], sulfurmycins[2] and aclacinomycins[3] producer. Usefulness of this process was evidenced by stereoselective glycosidation of racemic aklavinone to aclacinomycins A and B. So, it is worthwhile to study the glycosidation ability of this strain more in detail.

In this report we describe the growth phase dependency of glycosidation activity, time course of glycosidation and the influence of the amount of aglycone added with *S. galilaeus* OBB-111-848. In this experiment, ε-pyrromycinone[4] was used as a model substrate for glycosidation.

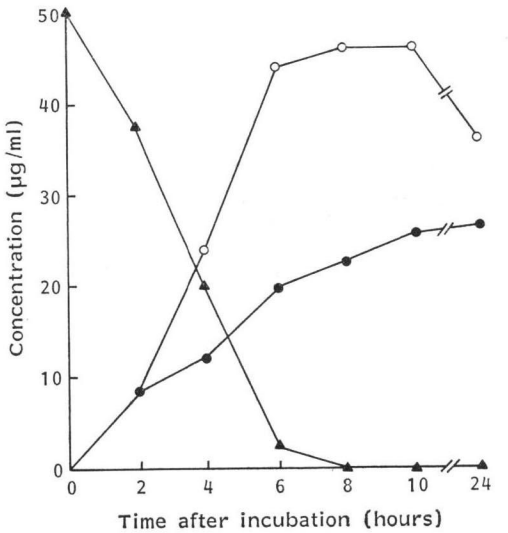
To a 100 ml of the culture of *S. galilaeus* OBB-111-848, prepared as described in the previous paper[1], 5 mg each of ε-pyrromycinone dissolved in 0.2 ml of dimethylsulfoxide was added on 1 ~ 6th day of pre-incubation. Each flask was harvested after 24 hours of glycosidation reaction by addition of 100 ml each of $CHCl_3$ and MeOH. The $CHCl_3$ layer was recovered and evaporated. The residue was dissolved in 0.2 ml of $CHCl_3$ and 20 μl of the sample was spotted on TLC plate (Silica gel plate $60F_{254}$, E. Merck & Co.) and developed (toluene - MeOH, 9: 1). Quantification of each component, cinerubin A[4], B[5] and ε-pyrromycinone was carried out using a chromatoscanner CS-910 (Shimadzu) at 490 nm. The results are shown in Table 1. When ε-pyrromycinone was added before the fourth day, that is, during the early and middle log phase of growth of *S. galilaeus* OBB-111-848, all ε-pyrromycinone added was glycosidated within 24 hours. On the other hand, when the aglycone was added after the fifth day, namely during the late log phase, about 80% of the aglycone remained unglycosidated. The ratio of cinerubins A and B was almost even regardless of the day of addition of the substrate.

Table 1. Change of glycosidation activity of *S. galilaeus* OBB-111-848.

Component	Cultivation days before ε-pyrromycinone addition					
	1	2	3	4	5	6
Cinerubin A	50*	46.2	50	54.1	9.6	8.4
Cinerubin B	50	53.8	50	45.9	9.6	12.3
ε-Pyrromycinone	0	0	0	0	80.8	79.3

* Relative amount.

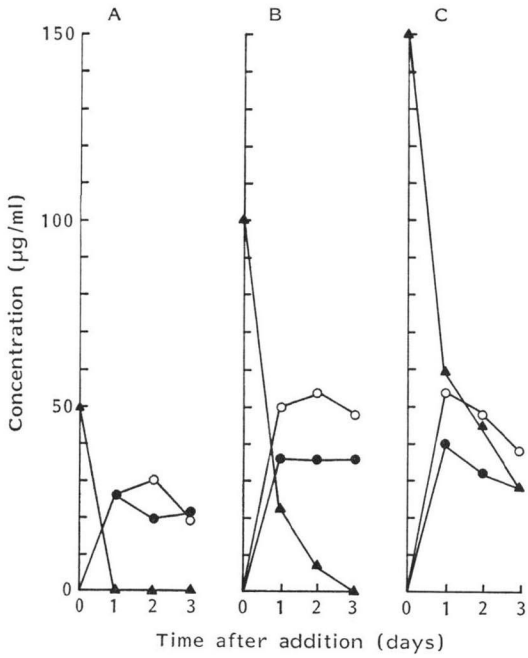
Fig. 1. Time course of glycosidation of ε-pyrromycinone by *S. galilaeus* OBB-111-848.
▲ ε-Pyrromycinone, ● cinerubin A, ○ cinerubin B.



Next, we examined the time course of the glycosidation process of *S. galilaeus* OBB-111-848. To the two-days culture of *S. galilaeus* OBB-111-848, 5 mg of ε-pyrromycinone was added. At 2, 4, 6, 8, 10 and 24 hours of glycosidation reaction, a portion (5 ml) of the broth was withdrawn and analyzed as described previously. The result is shown in Fig. 1. By 8-hour incubation after the addition of ε-pyrromycinone, the aglycone was converted to cinerubins A and B completely. The ratio of the two antibiotics changed during incubation; in early period of incubation, that is at 4, 6 and 8th hour, the ratio

Fig. 2. Influence of addition amount of ε-pyrromycinone for glycosidation by *S. galilaeus* OBB-111-848.

▲ ε-Pyrromycinone, ● cinerubin A, ○ cinerubin B.



of A to B was 1: 2 but it later changed to 1: 1.4 at 24th hour. The change of ratio of cinerubins A and B after the completion of glycosidation of ε-pyrromycinone suggested that the conversion of cinerubin B to cinerubin A or degradation of the component might have occurred.

The bioconversion of aclacinomycin A to aclacinomycin B *via* aclacinomycin Y was reported by OKI *et al.*[6]. According to the report, an aclacinomycins-negative mutant lacked this conversion activity. However, the same conversion was found with our strain, *S. galilaeus* OBB-111-848. Recently, we found that cinerubin B, auramycin B, sulfurmycin B and aclacinomycin B were converted to corresponding glycosides A by *S. galilaeus* OBB-111-848[7]. It was not found to be the reverse reaction of the bioconversion which was reported by OKI *et al.*[6]. So, it is postulated that the mutual conversion of cinerubins A and B occurred after completion of glycosidation of ε-pyrromycinone in the culture broth of *S. galilaeus* OBB-111-848.

Finally we examined the capacity of glycosidation of *S. galilaeus* OBB-111-848 by increasing the amount of ε-pyrromycinone added to the culture. To the two-days culture of *S. galilaeus* OBB-111-848, 5, 10 or 15 mg of ε-pyrromycinone was added. On the day 1, 2 and 3 after addition of ε-pyrromycinone, the sample was withdrawn and analyzed. The results are shown in Fig. 2-A, B and C. When 5 mg of ε-pyrromycinone was added (Fig. 2-A), ε-pyrromycinone disappeared within one day and slight degradation of cinerubins A and B was observed during the next two days of incubation. In case of addition of 10 mg of ε-pyrromycinone about twice as much as cinerubins A or B compared to the case of 5 mg of ε-pyrromycinone was produced. However, when 15 mg of ε-pyrromycinone was added, no increase of cinerubins A and B was observed. From these results, optimum amount for glycosidation by *S. galilaeus* OBB-111-848 was determined to be 10 mg for a 100 ml of culture.

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