Communications to the editor

EFFECT OF ACRIFLAVINE ON THE PRODUCTION OF β-LACTAMASE IN *STREPTOMYCES*

Sir:

It is known that the enzyme β-lactamase has a crucial role in bacterial resistance against β-lactam antibiotics. Some bacterial strains produce β-lactamases through a determinant on a plasmid[1], while in others the production of the enzyme is chromosomally mediated[2]. In a previous paper, we found that about 75% of the *Streptomyces* strains newly isolated from soil produced a significant amount of β-lactamase[3]. From the point of view of how the β-lactamase in *Streptomyces* is related to that produced by bacteria and what physiological role the β-lactamase in *Streptomyces* may have, it is interesting to know whether the production of β-lactamase in *Streptomyces* is determined by a plasmid or by the chromosome. In this paper we report the results of the effect of acriflavine, ethidium bromide and sodium dodecyl sulfate on the production of β-lactamase enzymes in two *Streptomyces* strains.

The medium of the shaking culture was composed of glucose, 1%; peptone (Polypeptone, Daigo Eiyo), 0.4%; yeast extract (Daigo Eiyo), 0.4%; KH_2PO_4, 0.2%; and K_2HPO_4, 0.4%. A 48-hour culture grown in the above medium on a rotatory shaking machine at 210 rpm was inoculated into a new medium of the same composition containing an appropriate amount of acriflavine[4], ethidium bromide[5] or sodium dodecyl sulfate[6] and was shake-cultured for 5~7 days at 27°C. A control in which no acriflavine, ethidium bromide or sodium dodecyl sulfate was present was cultured in the same way. After the incubation the mycelium from each culture was collected by centrifugation, washed with 0.85% NaCl and suspended in 0.85% NaCl. The mycelium was then cut by treatment for a few minutes with a vibrator and the suspension inoculated onto an agar medium composed of glucose, 1%; sodium asparaginate, 0.1%; asparagine, 0.05%; $MgSO_4 \cdot 7H_2O$, 0.05%; $CaCO_3$, 0.05%; K_2HPO_4, 0.1%; and agar, 2%. After incubation at 27°C for an appropriate period when the diameter of colonies was about 2 mm, the production of β-lactamase by the colonies was tested by the phenol red method. The phenol red reagent consisted of 1.2% agar, 5 ml; 0.1% phenol red adjusted to pH 9, 1 ml; and 10% ampicillin or benzylpenicillin, 0.5 ml per plate.

The results are summarized in Table 1. In the case of *Streptomyces* E750-3, 3 out of 529 colonies did not produce β-lactamase in the control culture, while 29 out of 1,055 colonies did not produce β-lactamase in a culture containing 40 μg acriflavine per ml. No morphological changes were detected in cured colonies. The production of β-lactamase by these colonies was checked by shaking culture in a medium composed of peptone, 3%; yeast extract, 0.2%; glycerol, 2.5%; $CaCO_3$, 0.6%; and silicone (KM-70, Shin Etsu Kagaku), 0.02%. Less than 0.2 units/ml of β-lactamases were produced in the cured colonies, and more than 54 units/ml of β-lactamases were produced in the original strain. (One unit was defined as the amount of β-lactamase enzyme which catalyzed the hydrolysis of 1 μmole of the substrate per minute.)

In a preliminary experiment with ethidium bromide at concentrations of 40, 20, 10, 5, 2 and 1 μg/ml all the colonies obtained after treatment with 10 μg ethidium bromide per ml as well as all the control colonies were found to produce β-lactamase. At 40 μg ethidium bromide per ml no growth of *Streptomyces* E750-3 was observed in the above medium. In the treatment with sodium dodecyl sulfate at a concentration of 25 μg/ml, 171 colonies out of 268 tested did not produce β-lactamase. However, in this case the properties of these colonies were clearly changed as indicated by an increase in pigment production in the slant cultures. As a result no further experiments were performed with these colonies. In the case of *Streptomyces* E756-1 all the colonies tested (over 500) produced β-lactamase in a control culture and 12 out of 624 colonies were thought not to produce β-lactamase at a first glance but in a few hours these colonies were found to produce β-lactamase enzyme in a culture containing 5 μg acriflavine per ml. The production of the enzyme in these colonies were also confirmed in shaken culture. About the same amounts of β-lactamase were produced in every case as in the original strain.

From the results described above, it seems

Table 1. Effect of acriflavine, sodium dodecyl sulfate and ethidium bromide on the production of β-lactamases in *Streptomyces* strains

Streptomyces strain	Reagents	Concentration of reagents (μg/ml)	No. of colonies tested	No. of colonies cured	Percentage of cured colonies
Streptomyces E750-3	Acriflavine	0	529	3	0.5
		40	1,055	29	2.8
	Sodium dodecyl sulfate	25	268	171[a)]	63.8
	Ethidium bromide	10	>500	0	0
Streptomyces E756-1	Acriflavine	0	>500	0	0
		5	624	12[a)]	1.9

a) See the text.

probable that the production of β-lactamase in *Streptomyces* E750-3 is controlled by a plasmid. The fact that the production of β-lactamase in *Streptomyces* E756-1 was not changed by acriflavine treatment does not necessarily mean that the ability to produce β-lactamase in this strain is not controlled by a plasmid. It is well known that the production of β-lactamase is controlled by a plasmid both in Gram-positive and Gram-negative bacteria[7]. Although *Streptomyces* is not a bacterium, the production of antibiotics[8] and of melanin pigment[9] in some *Streptomyces* has clearly been shown to be controlled by a plasmid. This means that at least some *Streptomyces* strains have a plasmid. From these facts, it is not unreasonable that the production of β-lactamase is controlled by a plasmid. It is known that an aminoglycoside antibiotic producing strain of *Streptomyces* produces an enzyme which has some role in the biosynthesis of this antibiotic and has a close relationship with the same antibiotic-inactivating enzyme produced by bacteria[10], and that the plasmid SCP 1 determines both the production of and resistance to the same antibiotic in *Streptomyces coelicolor* A3(2)[11]. It has been shown in a previous paper[3] that the productivity of β-lactamase is not directly related to the resistance against β-lactam antibiotics in *Streptomyces*. Thus, the exact role of β-lactamase in *Streptomyces* is not known at present. However, if the production of β-lactamase is controlled by a plasmid, it is interesting in relation to the possible origin of plasmid-borne genes conferring resistance to β-lactam antibiotics in Gram-positive and -negative bacteria. However, further studies are necessary for the confirmation of the implication of the plasmid in the production of β-lactamase in *Streptomyces*.

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