STUDIES ON PENICILLINASE INHIBITORS PRODUCED BY MICROORGANISMS

Sir:

The drug resistance of pathogenic bacteria is a very important problem in chemotherapy. Approaches[1,2,3] to this problem have been studied. It is known that the mechanism of penicillin resistance on bacteria is mainly inactivation of penicillin (PC) by penicillinase (PCase) derived from PC-resistant strains. We established a screening method relating to PCase inhibitors and investigated the PCase inhibitory activity of metabolites of various microorganisms, *i.e.* bacteria, fungi and streptomycetes. Activity was observed only in culture filtrates of certain streptomyces species. *Streptomyces gedanensis*, *Streptomyces olivaceus*, *Streptomyces* No. OS-3301 and *Streptomyces* No. OS-3391 were found to be the strains producing PCase inhibitors.

The PCase-producing strain, *Staphylococcus aureus* FS-1277, was inoculated into 100 ml medium containing 1.0 % Polypeptone and 0.5 % NaCl in a 500 ml flask and cultivated on a reciprocal shaking machine at 27°C. A 48-hour culture was transferred into 20 liters of medium containing 0.2 % glucose, 0.2 % KH_2PO_4, 0.01 % $MgSO_4$, 1.0 % yeast extract and 200 u/ml PC-G as a PCase inducer in a 30 liter jar fermentor and the fermentation was carried out at 27°C for 48 hours. This exo-PCase (FS-1277 PCase) was obtained as crude powder by salting-out with ammonium sulfate, dialysis and lyophilization. The screening procedure of PCase inhibitors was as follows: A mixture consisting of 0.25 ml of FS-1277 PCase solution (20 u/ml in M/10 phosphate buffer, pH 7.0) and 0.25 ml of culture filtrate of various microorganisms was pre-incubated at 37°C for 10 minutes, and 0.5 ml of PC-G solution (200 u/ml in M/10 phosphate buffer, pH 7.0) was added. The whole was incubated at 37°C for 10 minutes and the reaction was stopped by heating at 80°C for one minute. PCase inhibitory activity was estimated by measuring residual PC contents of the reaction mixture with paper disc method using *S. aureus* FDA 209 P as a test organism. PCase inhibitory titer was calculated as follows: The reaction mixture containing 100 u/ml PC-G and 5 u/ml PCase was used and 50 % inhibition of PCase activity was defined as one unit of PCase inhibitory activity.

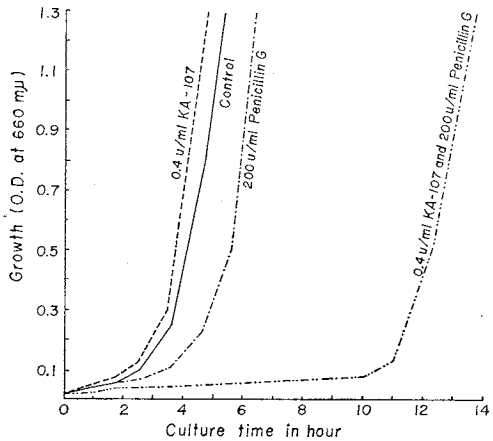
As a result, PCase inhibitory activity of the culture filtrate of *S. gedanensis* ATCC 4880 KA-107 was observed to be the highest. Production, purification and various properties of the effective substance (KA-107) were studied extensively. The strain was cultivated in a 30 liter jar fermentor in 20 liters of medium containing 2 % glucose, 0.5 % peptone, 0.5 % meat extract, 0.3 % dried yeast and 0.3 % $CaCO_3$ (pre-sterile pH 7.0) at 27°C. KA-107 was already produced after 20 hours of cultivation and the production reached a maximum after 45~55 hours. After filtration through a bed of Celite 545, KA-107 in culture filtrate was obtained as brownish crude powder by salting-out with 80 % saturated ammonium sulfate, dialyzing and lyophilizing. This powder was dissolved in water, applied on DEAE-cellulose column pre-equilibrated with 0.01 M phosphate buffer, pH 7.0. The column was washed with 0.1 M NaCl in 0.01 M phosphate buffer (pH 7.0) and developed with 0.3 M NaCl in 0.01 M phosphate buffer (pH 7.0). The active fraction was precipitated with 35~55 % saturated ammonium sulfate, dialyzed in 0.01 M phosphate buffer (pH 7.0) and lyophilized to yield white powder. KA-107 was then purified by Sephadex G-75 column chromatography. Based on analytical data of KA-107 at this purification step, KA-107 was considered to be a protein.

The effect of PC and PCase inhibitor KA-107 on the growth of PC-resistant *Staphylococcus aureus* FS-1277 was studied under the following conditions. FS-1277 culture (0.1 ml) in stationary growth phase was inoculated into 5 ml of a medium containing 0.1 % glucose, 0.2 % yeast extract, 0.3 % NaCl, 0.0011 % $CaCl_2$, 0.0044 % KH_2PO_4, 0.0095 % $MgCl_2$, 2.5 ml/liter of 1 N NaOH and various concentrations of PC-G or semisynthetic PC's with or without KA-107 and the whole was incubated with shaking at 30°C. Bacterial growth was followed

by measuring the optical density at 660 mμ with Coleman spectrophotometer.

As shown in Fig. 1, the growth of FS-1277 was not inhibited either in the presence of 200 u/ml PC-G or 0.4 u/ml of KA-107 independently. However, the growth of this strain was observed to be inhibited by the combined use of both agents. This effect was also found when 100 μg/ml ampicillin or phenethicillin, which could be inactivated by FS-1277 PCase, was used in the place of PC-G. The growth of FS-1277 strain was inhibited in the presence of 1 μg/ml cloxacillin, 0.5 μg/ml dicloxacillin, 1 μg/ml oxacillin or 5 μg/ml methicillin. FS-1277 PCase could not inactivate these semisynthetic PC's. The results obtained by the growth experiments indicated a substrate specificity of FS-1277 PCase to various types of semisynthetic PC's.

Fig. 1. Effect of penicillin G and KA-107 on the growth of *Staphylococcus aureus* FS-1277.



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