3″-PHOSPHORYLDIHYDROSTREPTOMYCIN PRODUCED BY THE INACTIVATING ENZYME OF *ERWINIA CAROTOVORA*

Sir:

Phytopathogenic bacteria resistant to antibiotics are isolated from diseased plants with increasing frequency. *Pseudomonas lachrymans* is known to be a plant-pathogen which causes cucumber angular leaf spot and the mechanism of dihydrostreptomycin(DHSM)-resistance is found to be due to streptomycin 3″-phosphotransferase[1,2].

We isolated streptomycin(SM)-resistant *Erwinia carotovora* from diseased plants in various localities of Japan[3]. These strains were resistant to SM, but susceptible to kanamycin, tetracycline, chloramphenicol, gentamicin, and spectinomycin. Four SM-resistant *E. carotovora* strains were randomly selected and the biochemical mechanisms of SM-resistance of these strains were investigated.

Heart infusion broth (HI, Eiken Chemical Co. Ltd., Tokyo) was used for liquid culture. Bacteria were inoculated in HI broth and shaken at 35°C. After 6 hours of incubation, cells were harvested by centrifugation. The S-30 fraction, the supernatant of 30,000 *g* centrifugation, was prepared as described in the previous paper[4]. Incubation reaction was carried out at 37°C for 1 hour, and then stopped by heating in boiling water for 1 minute. Antibiotic activity remaining in the reaction mixture was determined by bioassay using *Bacillus subtilis* ATCC 6633 as a test organism. Four *E. carotovora* strains inactivated SM. Dihydrostreptomycin (100 mg) was inactivated at 37°C in a reaction mixture containing 775 mg of disodium ATP, 50 mg of magnesium acetate, 63 ml of S-30 fraction from E7135 strain (43 mg of protein/ml) and 27 ml of 0.5 M tris-NCl buffer (pH 8.0). The total volume was 90 ml. After incubation for 6 hours, the reaction mixture was heated in boiling water bath for 5 minutes to stop the reaction. The solution was centrifuged and the supernatant was passed through a column of Amberlite CG-50 (NH_4^+) form, 50 ml. After washing the column with 1,000 ml of distilled water, the inactivated DHSM was eluted with 0.5 N aqueous ammonia. The eluate which gave positive in both SAKAGUCHI[5] and HANES reactions[6] was collected and lyophilized. The powder was subjected to the rechromatography on Amberlite CG-50 (NH_4^+ form, 50 ml) and eluted with 0.1 N aqueous ammonia. The inactivated DHSM fractions were collected and passed through a column of Sephadex G 10 (2 × 64 cm). Purified inactivated DHSM (63.3 mg) was obtained as a white powder.

On high-voltage paper electrophoresis under 3,500 volts for 20 minutes using formic acid - acetic acid - water (25: 75: 900 in volume), the inactivated DHSM and the authentic samples of DHSM 3″-phosphate prepared from both *Pseudomonas aeruginosa* Tl-13 and *P. lachrymans* N-7554 moved toward the cathode 6.8 cm, while DHSM moved 11.0 cm.

The pmr spectrum of the inactivated DHSM in D_2O (2.5 mg/0.3 ml) showed that the 3″-H signal was shifted to a lower field by approximately 0.25 ppm than that of DHSM. These chemical shifts were identical with that of DHSM 3″-phosphate reported by NAGANAWA *et al.*[7]

Conjugal transferability of SM-resistance in 4 *E. carotovora* strains was examined using *E. coli* ML 1410 (*met* F^- nalidixic acid-resistance) as a recipient. A strain E7135 could transfer its resistance to SM. The transfer frequency per donor cells was 9×10^{-7} after 3 hours of incubation. But the remaining 3 strains could not transfer their SM resistance to ML1410 in the same conditions. The genetic properties of this R plasmid will be described elsewhere.

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