EFFICACY OF BLEOMYCIN IN THE PROTECTION OF RICE SHEATH BLIGHT DISEASE

MASARU SHIMURA, TETSURO WATANABE, TAKAO OHASHI, TAKASHI SHOMURA, TARO NIIDA and YASUHARU SEKIZAWA

Research Laboratories, Meiji Seika Kaisha, Ltd., Kohoku-ku, Yokohama, Japan

HAMAO UMEZAWA

Institute of Microbial Chemistry, Shinagawa-ku, Tokyo, Japan

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In the course of screening against *Pellicularia sasakii*, a fermentation broth of E-327, a species of *Streptomyces*, was found to be effective against rice sheath blight disease.

The active substances resembled the bleomycin group by thin-layer chromatography and were purified by methods reported for these antibiotics[1], using activated charcoal instead of alumina. Five antibiotics were separated and identified as bleomycin A5, A6, B2, B4 and B6.

They were tested by the cylinder plate method and by pot tests against rice sheath blight, with the results shown in Fig. 1 and in Table 1. As shown in Fig. 1, bleomycin A5, A6, B2, B4 and B6 showed strong inhibitory effect against *P. sasakii*, with B2 having the most activity. Against *Bacillus subtilis*, bleomycin A5, B2 and B4 showed approximately the same activity. As shown in Table 1, all bleomycins produced by E-327 strain and commercially available bleomycin (more than 50 % of A2 content) were effective against rice sheath blight in the pot test. They were superior to the reference disinfectant Monzet in this test system. No signs of foliar burning or other phytotoxicity were observed.

The antimicrobial activities of bleomycin against many phytopathogenic microorganisms have been reported[4], but this is the first report of the *in vitro* and *in vivo* activity against *P. sasakii*. Bleomycin exhibits strong protective effect against rice sheath blight with low toxicity for the rice plant.

Table 1. Protection against rice sheath blight disease of bleomycin fractions

Sample	Concentration (ppm)	Protective value (%)
Bleomycin (commercial)	20	91
〃	30	95
Bleomycin A5	20	86
〃 A6	〃	83
〃 B2	〃	86
〃 B4	〃	68
〃 B6	〃	51
Monzet	(1/2500)	58
Blank		0

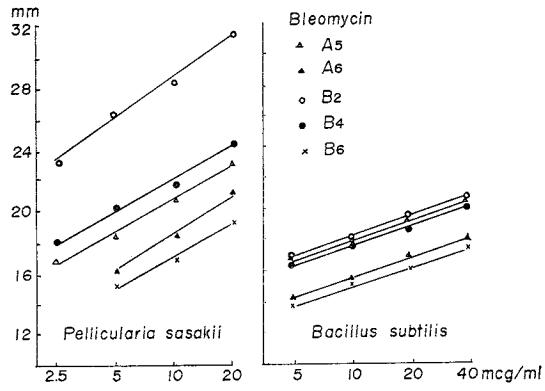
The rice plants were grown in pots (9 cm in diameter) in a phytotron under sunlight at 28℃ for 2 months and 50 ml of aqueous solution of sample was sprayed per one test unit consisted of three pots. A strongly virulent *P. sasakii** was inoculated to the leaf sheath[2,3] immediately after the sprayed test solution was air-dried. The pots were covered with filter paper and vinyl film tube, kept at 30℃ for 5 days and the symptomatic spots formed were counted.

Protective value was calculated according to the following formula.

$$\text{Protective value (\%)} = \left(1 - \frac{\text{Average spots of the treated}}{\text{Average spots of the non treated}}\right) \times 100$$

* Generous gift from Dr. M. HORI, Yamaguchi Agricultural Experimental Station, Ouchi, Yamaguchi.

Fig. 1. Antimicrobial activity of bleomycin fractions.



Assay plate for *P. sasakii* was prepared as following. At the center of the agar plate consisted of potato decoction medium containing 2 % glucose, agar disc (6 mm in diameter) taken from the 48 hours plate-culture of *P. sasakii* was placed. Aqueous solution of each bleomycin was added to the cylinder placed around the disc of *P. sasakii*.

Estimation of diameter of inhibitory zone (3 plates for each sample) was carried out after 48 hours incubation at 28℃ for *P. sasakii* and after 20 hours incubation at 37℃ for *B. subtilis*.

Each sample was the formate of individual bleomycin containing copper and each bleomycin fraction was pure by thin-layer chromatography.

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