キュウリ斑点細菌病菌(Pseudomonas lachrymans)の薬剤耐性

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Drug-resistance of Cucumber Angular Leaf Spot Bacterium, *Pseudomonas lachrymans* (Smith et Bryan) Carens

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矢野　博*・藤井　溥*・向　秀夫*・福安嗣昭**・渡辺哲郎**・関沢泰治**：
キュウリ斑点細菌病菌（*Pseudomonas lachrymans*）の薬剤耐性

Angular leaf spot of cucumber, caused by *Pseudomonas lachrymans* has become epidemic since many years ago, in every part of Japan. To facilitate chemical control of the disease, we have investigated the resistance of the causal bacterium to several antibiotics and $HgCl_2$. In this paper, results of experiments on minimal inhibitory concentration (MIC) of 13 antibiotics and Hg^{2+} against 165 isolates of *Pseudomonas lachrymans* were described. These isolates were collected from various districts of Japan, and the distribution pattern of drug-resistant bacteria was investigated.

Pseudomonas lachrymans isolates : During the period from 1973 to 1977, 165 isolates of *Pseudomonas lachrymans* were obtained from various districts of Japan. Among them, 110 isolates were generously provided by Dr. A. Ohuchi, National Institute of Agricultural Sciences.

Drugs : Following 13 antibiotics which had been commonly used for agricultural and medical purposes, and $HgCl_2$ were tested.

Dihydrostreptomycin (DHSM)	Kasugamycin (KSM)
Kanamycin (KM)	Oleandomycin (OL)
Chloramphenicol (CP)	Spectinomycin (SPC)
Gentamicin (GM)	Aminobenzylpenicillin (ABPC)
Carbenicillin (CBPC)	Oxytetracycline (OTC)
Novobiocin (NB)	Rifampicin (RFP)
Nalidixic acid (NA)	$HgCl_2$ (Hg^{2+})

Each drug except CP, RFP and NA, was dissolved in sterilized distilled water at the concentration of 8,000μg/ml, only DHSM was at 128,000μg/ml. CP and RFP were first dissolved in small amounts of propylene glycol and dimethyl sulfoxide respectively, and diluted by sterilized distilled water at the concentration of 8,000μg/ml. NA was dissolved in 0.1N NaOH (1/5 volume), then 4/5 volume sterilized distilled water was added to make the final concentration of 8,000μg/ml.

Medium : Peptone broth agar (PBA) of the following formula was used. For the multiplication of test bacteria, peptone broth (PB : only agar was excluded from

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PBA) was used.

Glucose 10g, 0.01M $CaCl_2$ 10 ml, 1N NaOH 2.5ml, Polypeptone 10 g, 0.1M $MgCl_2$ 10 ml, 0.1M KH_2PO_4 3.2 ml, NaCl 3 g, Agar 15 g, Distilled water 1000 ml.

Sensitivity test : For the determination of minimal inhibition concentration (MIC), agar streak method was applied on 2-fold serial dilution plates[1]. Namely, 1 ml of 2-fold serial dilutions of each drug was poured in a P-dish, 9 ml of melting PBA was added, thoroughly mixed and solidified. Test bacteria were cultured in PB for 24 hr, suspended in sterilized physiological saline at the concentration of 10^6 cells/ml, and 1 loopful of each suspension was streaked on dilution plates. MIC was investigated after incubation at 26~28 C for 24 hr.

Sensitivity distribution curves obtained from MIC values of 165 isolates were shown in Fig. 1.

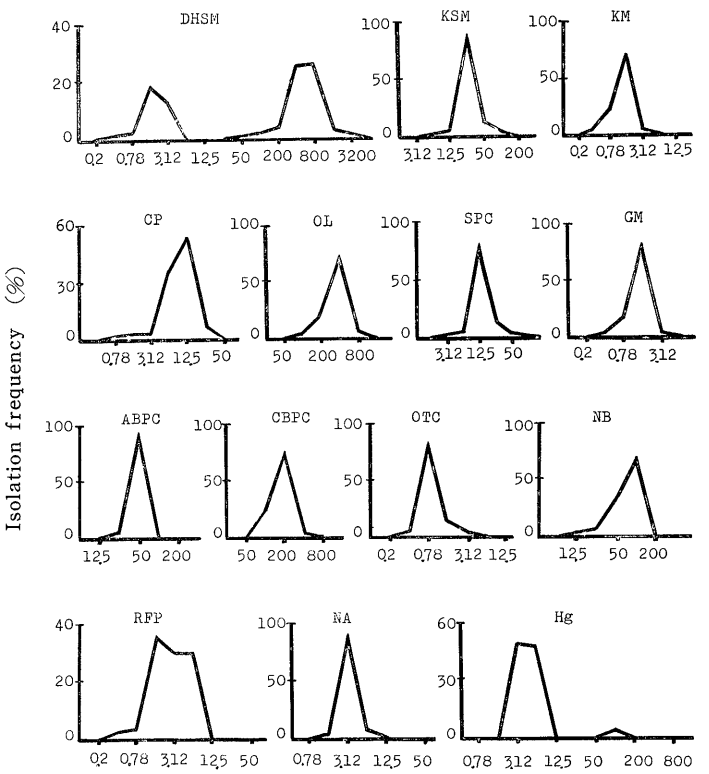

Fig. 1 Sensitivity distribution of 165 isolates of *Pseudomonas lachrymans* against various drugs.

Sensitivity to DHSM was shown by a two-peak curve as reported by Sakurai *et al* [2),3)], and 165 isolates were divided into 2 groups, sensitive group (1.56μg/ml peak) and resistant one (400~800μg/ml peak). Similar two-peak curve was also shown for Hg^{2+}, indicating sensitive (3.12~6.25μg/ml) and resistant (100μg/ml) bacterial groups.

As for other drug materials, however, normal distribution curves were shown. Peak MIC values were ; 0.78μg/ml for OTC, 1.56μg/ml for KM, GM, 1.56~6.25μg/ml for RFP, 3.12μg/ml for NA, 12.5μg/ml for CP, SPC, 25μg/ml for KSM, 50μg/ml for ABPC, 100μg/ml for NB, 200μg/ml for CBPC, and 400μg/ml for OL.

Table 1. Resistance pattern of *Pseudomonas lachrymans* to drugs.

Resistance pattern	No. of isolates (%)
DHSM, Hg^{2+}	6 (3.6)
DHSM	103 (62.4)
Sensitive[a)]	56 (33.9)
Total	165 (100)

a) Sensitivity was tested against DHSM, KSM, KM, OL, CP, SPC, GM, ABPC, CBPC, OTC, NB, RFP, NA and Hg^{2+}.

Table 2. Yearly changes in the frequncy of DHSM-resistant isolates of *Pseudomonas lachrymans*.

	1973~74	1975	1976	1977
No. of isolates tested	14	68	36	44
No. of DHSM-resistant isolates.	9	44	22	34
(%)	64.3	64.7	61.1	77.3

Table 3. Isolation frequency of DHSM-resistant *Pseudomonas lachrymans* in various districts of Japan (1973~77).

	No. of tested isolates	No. of DHSM-resistant isolates (%)
Iwate	5	2 (40)
Miyagi	3	3 (100)
Fukushima	15	15 (100)
Gunma	25	21 (84)
Saitama	34	17 (50)
Chiba	17	3 (17.6)
Tokyo	3	3 (100)
Kanagawa	25	25 (100)
Osaka	5	0 (0)
Hiroshima	10	10 (100)
Ehime	11	10 (90.9)
Kochi	3	0 (0)
Nagasaki	4	0 (0)
Miyazaki	5	0 (0)
Total	165	109 (66.1)

DHSM resistant isolates were 62.4% in ratio, and double-resistant ones against both DHSM and Hg^{2+} were 3.6%, as shown in Table 1.

It seemed that the DHSM resistant isolates gradually increased during the years from 1973 to 1977, though the tendency was not so evident.

As above mentioned, 165 *Pseudomonas lachrymans* isolates showed two peak distribution curves of MIC against DHSM and Hg^{2+}, some of which were double-resistant to both drugs.

Since nearly 20 years ago, increase of drug-resistant bacteria has been seriously taken into consideration in medical fields [4)]. Such drug-resistant bacteria were also found among normal flora or pathogenic bacteria of domestic animals[5),6),7)], cultured fish[8)], and plants[3),9)]. Importance of such drug-resistance of microorganisms will be much increased in the fields of medical-, animal-, fishery- and plant-science.

1) Ishiyama, S., Ueda, Y., Kuwabara, S., Kosakai, N., Koya, G., Konno, N., and Fujii, R. (1968). Chemotherapy 16(1) : 98-99.
2) Hasuda, K., Sakurai H. (1977). Medicine and Biology. 95(3) : 203-206.
3) Sakurai, H., Naito, H., and Fujita, S.(1976). J. Antibiotics 29 (11) : 1230-1236.
4) Mitsuhashi, S. (1975). Drug Action and Drug Resistance in Bacteria. 2. Aminoglycoside Antibiotics. University of Tokyo Press. pp.179-207.
5) Suzuki, K., Isogai, S., Hashimoto, H., and Mitsuhashi, S. (1967). Japan. J. Bacteriol. 22(3) : 146-150.
6) Suzuki, K., Isogai, S., and Hashimoto, H. (1968). *Ibid.* 23(6) : 419-422.
7) Suzuki, K., Isogai, S., and Hashimoto, H. (1970). *Ibid.* 25(3) : 145-148.
8) Aoki, T., Egusa, S., and Watanabe, T. (1972). *Ibid.* 27(6) : 762-767.
9) Sakurai, H. (1977). J. Pesticide Sci. 2(2) : 177-186.