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Yutaka Tanaka, Takashi Matsushima, and Mineko Ikeda : Growth Inhibition of Common Saprophytic Fungi by Various Antibiotics

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In recent years, many antibiotics have been isolated, but most of them are antibacterial and only comparatively few are antifungal. Among antifungal antibiotics, Actidione[1~3] and Candicidin[4,5] are well known and are used clinically to some extent. Attempts to isolate antifungal antibiotics have been directed to plants or human pathogenic fungi, and relatively little attention has been paid to common saprophytic fungi. Saprophytic fungi like Aspergillus and Penicillium are very important in microbial spoilage of foods and in other industrial fields. In this respect, effect of various kinds of antibiotics for many species of saprophytic fungi were tested and compared under the same experimental conditions.

Materials and Method

The 16 antibiotics used in this test were as follows : Actidione (Upjohn, Res. No. 9814~5), Filipin[6,7] (Upjohn, Res. No. 11,148, purity 94%), Candicidin (Merck, 54R2758), Rimocidin (Pfizer, purity 76%), Cathomycin Monosodium Salt (Merck, MK—77), Puromycin Hydrochloride (American Cyanamid, #3241B-77A), Anisomycin (Pfizer, purity 96%), Tyrothricin (Merck, 54R1598), Neomycin (Merck, 54R1553), Calcium Oxamycin (Merck, (MK-65) 55R2172), Tetracycline Hydrochloride (American Cyanamid, #5747-321), Pleocidin (Merck, 53R2748), Fungichromin (Merck, 54R5821), Trichomycin (Fujisawa, 2160 μ/mg.), Bacitracin (Merck, 54R1594), and Gramicidin S Sulfate (Merck, 54R5004).

One gram of each antibiotic was dissolved in 100 cc. of sterile distilled water or EtOH, depending on its property. Each stock solution was added to asparagine-dextrose-agar, just before pouring into plates, to make the final concentration of 100 p.p.m. The composition of asparagine-dextrose-agar was as follows :

L-Aspragine monohydrate ..... 2.0 g.	Zn (from $ZnSO_4$).....0.2 mg.
Dextrose.....25.0 g.	Mn (from $MnSO_4$) .....0.1 mg.
KH_2PO_4 ..... 1.0 g.	Cu (from $CuSO_4$).....0.05 mg.
$MgSO_4 \cdot 7H_2O$ ..... 0.5 g.	Mo (from $(NH_4)_6Mo_7O_{24}$).....0.05 mg.
Agar.....15.0 g.	Distilled water.....to make 1000 cc.
Fe (from $FeCl_3$) .....0.2 mg.	pH 6.0 (before autoclaving)

All fungi tested were stock cultures of National Hygienic Laboratory. As inocula, 7-day-old cultures on potato-dextrose-agar slant were used and inoculated as a point in the center of Petri plate.

The effect of antibiotics was evaluated by measuring the diameter of the colonies after 4, 7, or 10 days' incubation at 25°, since the fungi varied in their growth rate. Triplicate plates were used in each experiment. Two diameter measurments were made to obtain an average of the largest and smallest diameters of asymmetrical colonies. The avarages were then compared, in percentage, to diameters on asparagine-dextrose-agar with no added antibiotic.

Discussion and Summary

Among the 16 antibiotics tested, Filipin, Rimocidin, and Fungichromin were most effective and completely inhibited the growth of majority of fungi tested at the concen-

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Experimental Result

Antibiotics \ Test Fungi	*Rhizopus nigricans*	*Mucor* sp. No. 51	*Mucor* sp. No. 52	*Aspergillus restrictus*	*Aspergillus amstelodami*	*Aspergillus chevalieri*	*Aspergillus ruber*	*Aspergillus candidus*	*Aspergillus versicolor*	*Aspergillus niger*	*Penicillium citreo-viride*	*Penicillium citrinum*	*Penicillium commune*	*Penicillium cyclopium*	*Penicillium rugulosum*	*Penicillium purpurogenum*	*Penicillium islandicum*	*Paecilomyces varioti*	*Trichoderma viride*	*Helminthosporium sativum*	*Curvularia lanata*	*Cladosporium herbarum*	*Alternaria* sp. No. 27	*Fusarium* sp. No. 26	*Byssochlamys fulva*	*Chaetomium* sp. No. 55
Acti-dione	12	0	40	—	—	32	22	20	52	32	69	50	48	52	10	66	74	0	6	0	0	50	64	24	0	22
Filipin	9	0	0	—	—	0	0	0	0	0	0	0	0	33	0	0	26	0	2	0	0	0	0	0	0	6
Candicidin	89	23	45	—	—	68	74	71	71	83	88	63	55	86	40	91	100	50	35	21	47	90	36	76	42	89
Rimocidin	0	0	0	—	—	0	0	0	0	0	0	0	0	14	0	0	30	0	0	0	0	0	0	0	0	0
Cathomycin mono-Na salt	59	18	38	50	42	57	41	74	95	100	88	74	54	76	77	59	95	39	100	—	21	69	32	78	53	68
Puromycin-HCl	82	100	100	100	79	95	73	84	100	100	94	95	72	100	100	100	100	100	100	—	44	100	84	94	100	80
Anisomycin	100	100	78	90	90	91	73	95	90	100	81	100	86	71	100	78	100	89	88	—	68	56	59	78	53	65
Tyrothricin	79	31	13	—	—	72	61	67	—	78	88	83	77	76	100	57	67	38	47	17	26	40	22	—	19	26
Neomycin	100	69	17	100	100	100	94	100	67	100	100	96	97	89	100	—	100	88	100	71	92	96	100	77	100	100
Ca-oxamycin	100	100	100	100	95	93	90	95	100	100	94	100	100	96	100	100	100	100	100	100	93	100	90	100	100	90
Tetracycline-HCl	100	97	94	100	100	94	94	100	71	100	92	83	—	95	93	—	81	83	100	100	80	67	86	100	79	69
Pleocidin	100	100	83	—	—	8	0	19	—	46	31	44	0	76	60	63	78	0	53	10	0	0	16	—	0	15
Fungichromin	6	—	0	—	—	0	0	0	0	0	0	13	6	33	15	0	26	0	0	0	0	0	0	0	0	0
Trichomycin	0	20	21	0	0	20	12	50	81	19	39	65	0	39	0	—	69	12	12	100	0	75	19	37	0	46
Bacitracin	100	100	100	100	86	100	100	93	91	55	92	87	100	83	93	—	87	96	100	90	96	100	92	96	100	100
Gramicidin S sulfate	100	50	66	75	58	86	59	53	75	98	88	95	100	100	100	78	100	23	94	—	19	50	21	84	41	20

tration of 100 p.p.m. Actidione, Pleocidin, and Trichomycin had moderate inhibitory effect, but others were not effective. In general, no marked specificity of an antibiotic for certain species of fungi was observed. Among 26 species of fungi tested, *Penicillium islandicum* was the most resistant against all antibiotics and *Byssochlamys fulva* was the most susceptible. These fungi may be used as both extremes in sereening antifungal substances.

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Tsukasa Kuraishi: 4,5-Substituted Pyridazines. II.[1]

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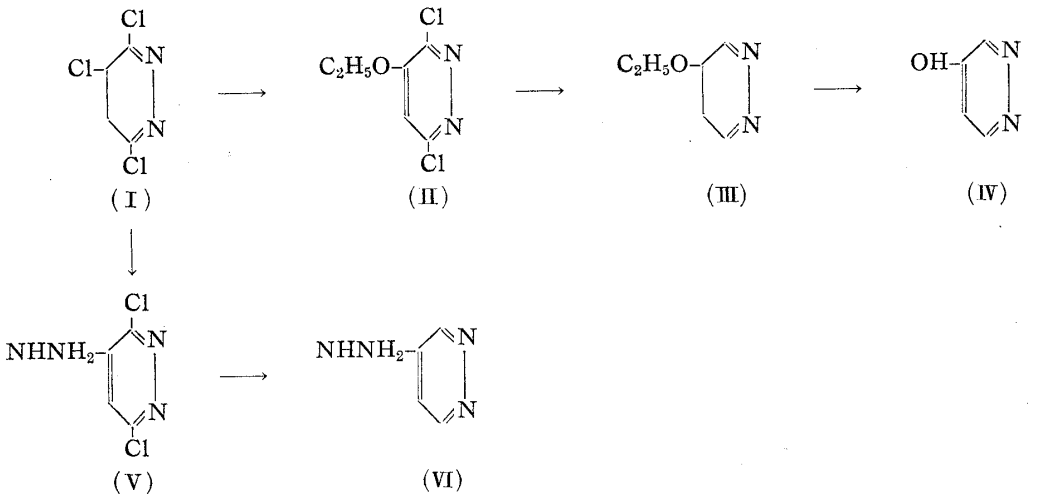
Although there have been many reports on the preparation of 4,5-substituted pyridazines in recent years, substitution reaction of these compounds have not been thoroughly described yet even in the case of a simple nucleophilic or electrophilic reagents because simple pyridazine derivertives are more inactive to usual reagents than pyridine derivertives.

To extend the preceeding studies on 4,5-substituted pyridazines, some of substitution reaction of chloropyridazines was examined.

The 4-substituted pyridazines, such as 4-amino-[2] and 4-methoxy-3,6-dichloropyridazine,[3] were obtained by nucleophilic attaks at the chlorine atom in 4-position of 3,4,6-trichloropyridazine. Similarly, 4-ethoxy- and 4-hydrazino-3,6-dichloropyridazines were obtained. Both reactions occurred at room temperature and the separated 4-hydrazino- and 4-ethoxy-3,6-dichloropyridazines were easily derived by catalytic reduction to 4-hydrazinopyridazine hydrochloride and 4-ethoxypyridazine, respectively.

4-Ethoxypyridazine was led to 4-hydroxypyridazine by refluxing in a sealed tube with an excess of anhydrous hydrobromic acid in glacial acetic acid at 120~125°.

These facts indicate that the chlorine atom in 4- or 5-position is more reactive to



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