Studies on the Antibiotic Action of the Bacterial Pigment, Iodinin

I. Antibiotic Spectra of Iodinin, and Its Reduced Form, 1,6-Phenazinediol*

Ikunosuke TANABE, Norihiko IIO,** Rikuro IMAMURA,*** Tomohiro MATSUMOTO,**** and Akira OBAYASHI*****

(*Laboratory of Applied Microbiology*)

(Received for publication August 31, 1973)

INTRODUTION

In 1962, two strains of marine brevibacteria, isolated from the sea water sample in the Sea to the East of Japan in the Pacific Ocean, were found to produce a large amount of iodinin extracellularly[1,2]. Iodinin, 1,6-phenazinediol-5,10-di-N-oxide, is one of the natural phenazine derivatives with an inhibitory action on the growth of various kinds of microorganism[3,4]. However the mode of action of iodinin has not been elucidated. Taxonomy, cultivation, and, iodinin production of *Brevibacterium stationis var. iodinino-faciens* were presented in the former reports[2,5]. Further investigations in our laboratory are to be directed to the biosynthesis and to the physiological activity of iodinin and 1,6-phenazinediol; and at the same time, the elucidation of the phylogenic meaning in the production of these pigments is expected.

This report describes the antibiotic spectra of iodinin, and 1,6-phenazinediol.

MATERIALS AND METHODS

Organisms. One hundred and thirty strains employed in this investigation were supplied from the Institute of Applied Microbiology, University of Tokyo (Abbreviation: IAM), the Institute for Fermentation, Osaka (Abbreviation: IFO), and the culture collection of this laboratory. They are shown in Table 1 and 2.

Culture media. Each strain was cultivated in one of the following media.

1. Nutrient broth for aerobic bacteria[6].

---

* This report is made a part of the one presented at the Annual Meeting of the Agricultural Chemical Society of Japan in 1970. Abstracts, p. 320.

** Present address: Shôwa Chemical Co., Ltd., Suita Plant, Osaka.

*** Present address: Meiji Milk Products Co., Ltd., Ryôgoku Plant, Tokyo.

**** Present address: Sanraku Ocean Co., Ltd., Tsukuda Plant, Osaka.

***** Present address: Takara Shuzo Co., Ltd., Central Research Laboratories, Shiga-ken.

Table 1. Antibiotic spectrum of iodinin.

organism	iodinin added (μg/ml)	inhibition (%)*	iodinin recovered (μg/ml)
(1) Bacteria			
Bac. sphaericus IFO 3341	1	+	
Bac. natto IFO 3339	1	+	
Bac. firmus IFO 3330	2	+	
Bac. circulans IFO 3329	1	+	
Bac. subtilis var. niger IFO 3108	4	+	
Bac. cereus var. mycoides IFO 3015	4—6	+	
Bac. subtilis IFO 3007	1	+	
Bac. megaterium IFO 3003	1—2	+	
Bac. megaterium IAM 1030	5	+	
Cor. equi IAM 1038	30	+	5.45
Br. st. var. iod. P_0-363-21	30	+	6.90
Achr. guttatus P_0-9	30	+	2.00
Staph. aureus IAM 1011	30	$+_{24}$	4.40
Br. ammoniagenes IAM 1641	30	$+_{24}$	19.40
Br. st. var. iod. mutant P_0-363-41	30	$+_{24}$	1.88
Bac. cereus IFO 3001	30	—	0.84
Ps. azotogena	30	—	1.0
Ps. chlororaphis IFO 3904	30	—	10.90
Ps. iodinum IFO 3558	30	—	18.60
Ps. aeruginosa Tr-11	30	—	18.40
Ps. aeruginosa Pk-199	30	—	14.90
E. coli IAM 1016	30	—	16.90
(2) Lactic acid bacteria			
Lac. acidophilus IFO 3831	10	+	
Lac. brevis IFO 3960	10	+	
Lac. plantarum 11	10	—	4.61
Leuc. mesenteroides B07	10	+	
Ped. acidilacti IFO 3076	10	+	
Lac. plantarum K-2	10	+	
Lac. sake O12	10	+	5.28
Streptococcus sp. KK-1-1	30	—	12.10
(3) Actinomycetes			
St. antibioticus IFO 3117	10	+	
St. olivaceus IFO 3152	10	+	
St. aureofaciens IFO 3305	10	+	
St. fradiae IFO 3360	10	+	
St. albus IFO 3418	10	+	
St. griseus IAM 0084	10	+	
St. lavendulae IAM 0009	10	+	
Streptosporangium roseum IAM 0119	30	+	
Myc. avium IFO 3082	30	+	2.10
Myc. smegmatis IFO 3083	30	+	0.48
Myc. phlei IFO 3158	10	+	
Nocardia asteroides IFO 3384	10	+	
Nocardia gardneri IFO 3385	10	+	
St. phaeochromogenes IFO3105	30	70	0.60
St. verne IFO. 3112	30	50	6.00
Nocardia corallina IFO 3338	30	40	3.20
St. ruber IFO 3110	30	—	5.56

organism	iodinin added (μg/ml)	inhibition (%)*	iodinin recovered (μg/ml)
(4) Molds			
Pen. roqueforti IFO 4622	10	+	
Pen. chrysogenum IFO 4626	10	+	0.84
Pen. funiculosum IFO 5857	10	+	
Pen. decumbens IFO 6093	10	+	
Pen. nigricans IFO 6103	10	+	
Asp. kawachii IFO 4308	30	+	
Asp. chevalieri IFO 4334	30	+	
Mortierella pusilla var. vinacea IFO 6223	10	+	
Tilachlidium humicola IAM 5025	10	+	
Pullularia pullulans IAM 5055	10	+	
Mortierella vinacea FRI Sz-1	10	+	
Hormoderum pedrosoi IAM 17-1	30	+	
Rh. japonicus IFO 4758	30	+	6.64
Zygorhynchus moelleri IAM 6234	30	+	
Polystictus sanguineus IAM 9005	30	+	
Asp. flavus IFO 4053	30	50	1.30
Asp. oryzae var. microsporus IFO 4220	30	70	2.00
Asp. sojae IFO 4244	30	70	1.57
Asp. oryzae var. magnasporus IFO 4050	30	75	1.40
Asp. luchuensis IFO 4281	30	80	
Asp. awamori IFO 4314	10	85	
Asp. flavus IAM 2007	10	90	3.40
Asp. oryzae IFO 2024	30	75	1.60
Asp. niger ATCC 6275	30	70	3.26
Asp. niger FRI 12	30	60	
Asp. niger FRI Sz-2	30	90	
Asp. clavatus IAM 2002	10	50	4.25
Cephalosporium acremonium IAM 5027	10	90	
Rh. oryzae IFO 4707	10	70	6.64
Asp. tamarii IFO 4099	30	—	2.02
Asp. awamori var. fumeus IFO 4122	30	—	5.04
Asp. oryzae var. globosus IFO 4214214	30	—	1.64
Asp. chevalieri IFO 4298	30	—	9.99
Asp. usamii IFO 4388	30	—	2.77
Asp. oryzae IFO 5239	30	—	5.12
Asp. usamii mut. shiro-usamii IFO 6082	30	—	4.30
Asp. nidulans IAM 2006	30	—	7.75
Botrytis cinerea IAM 5126	30	—	10.00
Fusarium moniliforme IAM 5062	30	—	13.60
Rh. delemar IFO 4698	30	—	5.81
Rh. javanicus var. kawasakiensis IFO 4801	30	—	4.80
Rh. javanicus IFO 5441	30	—	8.70
Rh. nigricans IAM 6070	30	—	12.50
Mucor javanicus IAM 6087	30	—	5.90
(5) Yeasts			
Schiz. pombe IFO 0346	10	+	
Sp. salmonicolor IFO 1038	10	+	
Kloeckera apiculata IAM 4018	10	+	
Pichia membranaefaciens IAM 4025	10	+	
Can. utilis IAM 4215	30	$+_{24}$	5.10

organism	iodinin added (μg/ml)	inhibition (%)*	iodinin recovered (μg/ml)
Can. mycoderma IAM 4564	30	$+_{24}$	4.60
Can. tropicalis IAM 4862	30	$+_{24}$	9.68
Crypt. neoformans IAM 4514	30	$+_{24}$	9.40
H. anomala IAM 4213	30	$+_{24}$	8.00
S. cerevisiae IAM 4428	10	$+_{24}$	9.20
Can. utilis IAM 4220	30	—	8.45
Can. albicans IAM 4888	30	—	7.97
S. cerevisiae IAM 4702	30	—	7.20
(6) Microalgae...(in light)			
Chlamydomonas sp. IAM C-21-1	10	+	12.60
Scenedesmus basilensis IAM C-66-1	10	+	9.20
Chlorela vulgaris Al-ly-3	10	+	
Chlorela vulgaris Al-1-3	10	+	11.60
Chlamydomonas sp. Al-5-2	10	+	4.22
Chlorella vulgaris Al-16	10	+	9.20
Chlorella vulgaris Al-28	10	+	11.60
Scenedesmus obliquus Al-55 (in darkness)	10	+	8.56
Chlamydomonas sp. IAM C-21-1	30	—	2.82
Scenedesmus basilensis IAM C-66-1	30	—	4.77
Chlorella vulgaris Al-ly-3	30	—	2.50
Chlorella vulgaris Al-1-3	30	—	3.12
Chlamydomonas sp. Al-5-2	30	—	3.59
Chlorella vulgaris Al-16	30	—	4.22
Chlorella vulgaris Al-28	30	—	2.42
Scenedesmus obliquus Al-55	30	—	2.89

* +: Complete growth inhibition. $+_{24}$: Growth inhibition continued for 24 hr.
—: No inhibition.

2. Sea water medium for marine bacteria (G-55)[5].
3. Yeast extract-malt extract medium for actinomycetes[7].
4. YPAG medium for lactic acid bacteria, composed of: yeast extract, 5 *g*; polypepton, 10 *g*; glucose, 10 *g*; sodium acetate, 10 *g*; deionized water, 1 *l*; *pH* 6.8.
5. Malt extract-nutrient broth for mass culture of lactic acid bacteria, composed of: malt extract, 25 *g*; beef extract, 3 *g*; polypepton, 5 *g*; sodium chloride, 3 *g*; deionized water, 1 *l*; *pH* 7.0.
6. Potato glucose broth for yeasts and molds[8].
7. Mineral salts solution for microalgae, composed of: yeast extract, 0.1 *g*; potassium nitrate, 2 *g*; A_3- solution (H_3BO_3, 2.86 *g*, $ZnSO_4 \cdot 7H_2O$, 0.222 *g*, and $CuSO_4 \cdot 5H_2O$, 0.79 *g* in 1 *l* of deionized water), 1.0 *ml*; mineral salts solution[6], 1 *l*; *pH* 7.2-7.4.

Only microalgae were cultivated in the following two ways, the first was done in the mineral salts solution for microalgae in light and the second, in the medium supplemented with glucose (1 %) in darkness.

Preparation of the pigments. Iodinin was prepared from the culture of *Brevibacterium stationis* var. *iodininofaciens* Po-363-21, and 1,6-phenazinediol was prepared by the reduction of iodinin with sodium hydrosulfite, according to the method of TANABE and OBAYASHI[5]. The pigment was sterilized by ethanol, dried up in a vacuum and dissolved

Table 2. Reaction of microorganisms with 1,6-phenazinediol

organisms	1.6-phenazine-diol added (μg/ml)	inhibition (%)	1,6-phenazine-diol recovered (μg/ml)
(1) Bacteria			
Aeromonas. hydrophila IAM 1018	30	−	13. 20
Agrobacterium. tumefaciens IAM 1037	30	−	9. 00
Arth. simplex IAM 1660	30	−	8. 39
Bac. alvei IFO 3343	10	+	1. 86
Bac. cereus IFO 3001	30	−	
Bac. cereus IAM 1029	30	−	2. 10
Bac. cereus. var. mycoides IFO 3015	30	−	12. 80
Bac. circulans IFO 3329	10	+	3. 67
Bac. firmus IFO 3330	30	−	8. 35
Bac. megaterium IAM 1030	30	−	7. 42
Bac. megaterium IFO 3003	10	−	1. 09
Bac. natto IFO 3339	30	−	2. 16
Bac. sphaericus IFO 3341	30	−	9. 38
Bac. subtilis IFO 3007	30	−	3. 47
Bac. subtilis var. niger IFO 3108	30	−	2. 74
Br. ammoniagenes IAM 1641	30	−	
Cor. equi IAM 1038	30	−	
E. coli IAM 1016	30	−	1. 09
E. coli IFO 3806	30	−	2. 34
Microbac. flavum IAM 1642	30	−	15. 00
Micrococ. luteus IAM 1097	30	−	12. 10
Proteus vulgaris IAM 1025	30	−	12. 90
Ps. aeruginosa P_0-199	30	−	
Ps. aeruginosa Tr-11	30	−	2. 10
Ps. aureofaciens IFO 3521	30	−	
Ps. chlorophis IFO 3904	30	−	
Ps. iodinum IFO 3558	30	−	0. 78
Ps. ovalis IAM 1002	30	−	11. 30
Sar. lutea IAM 1099	30	−	10. 90
Ser. marcescens IAM 1021	30	−	11. 40
Staph. aureus IAM 1011	30	−	9. 77
Achr. guttatus P_0-9	30	−	
Br. stationis var. iod. P_0-363-21	30	−	13. 60
Br. stationis var. iod. mutant P_0-363-41	30	−	4. 84
Ps. azotogena	30	−	
(2) Lactic acid bacteria			
Lac. acidophilus IFO 3831	10	+	
Leuc. mesenteroides BO7	10	+	
Lac. sake O12	30	+	4. 97
Ped. acidilactici IFO 3076	10	+	
Lac. plantarum 11	30	−	3. 91
Lac. plantarum K-2	30	−	4. 22
Streptococcus sp. KK-1-1	30	−	9. 39
(3) Actinomycetes			
Myc. smegmatis IFO 3083	30	−	1. 64
Noc. asteroides IFO 3384	30	−	
Noc. corallina IFO 3338	30	−	
Noc. gardneri IFO 3385	30	−	
Str. griseus IAM 0084	30	−	3. 44

organisms	1,6-phenazinediol added (μg/ml)	inhibition (%)	1,6-phenazinediol recovered (μg/ml)
Str. fradiae IFO 3360	30	—	
Str. phaeochromogenes IFO 3105	30	—	
Str. verne IFO 3112	30	—	1.09
Myc. avium IFO 3082	10	40	
Myc. phlei IFO 3158	10,30	20	
Str. albus IFO 3418	10,30	80	
Str. antibioticus IFO 3117	10	+	
Str. aureofaciens IFO 3305	10,30	+	
Str. coelicolor IFO 3114	10	+	3.12
Str. ruber IFO 3110	30	+	
Str. lavendulae IAM 0009	10	+	2.90
Str. olivaceus IFO 3152	10,30	+	0.70
Streptosporangium roseum IAM 0119	10	+	
(4) Molds			
Asp. clavatus IAM 2002	30	—	
Asp. flavus IFO 4053	30	—	
Asp. flavus IAM 2007	30	—	
Asp. luchuensis IFO 4281	30	—	
Asp. nidulans IAM2006	30	—	
Asp. niger ATCC 6275	30	—	
Asp. niger FRI Sz-2	30	—	
Asp. oryzae IAM 2024	30	—	
Asp. oryzae IFO 5239	30	—	
Asp. oryzae var. microsporus IFO 4220	30	—	
Asp. oryzae var. magnasporus IFO 4050	30	—	
Asp. oryzae var. globosus IFO 4214	30	—	2.50
Asp. sojae IFO 4244	30	—	
Asp. tamarii IFO 4099	30	—	
Asp. usamii IFO 4388	30	—	
Pen. camemberti IFO 4626	30	—	
Pen. roqueforti IFO 4622	30	—	
Mucor javanicus IFO 6087	10	—	
Rhi. javanicus IFO 5441	30	—	0.55
Rhi. javanicus var. kawasakiensis IFO 4801	30	—	
Rhi. nigricnas IAM 6070	30	—	
Polystictus versicolor IAM 9018	30	—	
Asp. awamori IFO 4314	30	30	
Asp. awamori var. fumeus IFO 4122	30	30	
Asp. kawachii IFO 4308	10,30	50	
Asp. niger FRI 12	30	30	0.70
Asp. usamii mut. shiro-usami IFO 6082	30	20	
Botrytis cinerea IAM 5126	10,30	70	
Cephalosporium acremonium IAM 5027	10,30	70	
Neurospora crassa IFO 6660	10,30	70	
Rhi. delemar IFO 4698	30	30	
Asp. chevalieri IFO 4334	10	(+)*	
Fusarium moniliforme IAM 5062	10,30	(+)	
Hormodendrum pedrosoi IAM 17-1	10,30	(+)	
Pen. camemberti IFO 5855	30	(+)	
Pen. claviforme IFO 5740	30	(+)	
Pen. decumbens IFO 6093	10	(+)	
Pen. funiculosum IFO 5857	10	(+)	
Pullularia pullulans IAM 5055	10	(+)	
Tilachlidium humicola IAM 5025	10	(+)	

organisms	1,6-phenazine-diol added (μg/ml)	inhibition (%)	1,6-phenazine-diol recovered (μg/ml)
Asp. chevalieri IFO 4298	10	+	
Mortierella vinacea FRI Sz-1	30	+	
Mortierella pusilla var. vinacea IFO 6223	10	+	
Pen. nigricans IFO 6103	10	+	
Rhi. javanicus IFO 4758	30	$+_w$	0. 62
Rhi. oryzae IFO 4707	10	+	
Zygorhynchus moelleri IAM 6234	30	+	
Polystictus sanguineus IAM 9005	10	+	
(5) Yeasts			
Ca. albicans IAM 4888	30	—	
Ca. mycoderma IAM 4564	30	—	
Ca. tropicalis IAM 4862	30	—	
Ca. utilis IAM 4215	30	—	
Ca. utilis IAM 4220	30	—	
Crypt, neoformans IAM 4514	30	—	2. 50
Endomyces magnusii IAM 4754	30	—	
H. anomala IAM 4213	30	—	2. 34
K. apiculata IAM 4018	10	—	
Sacch. cerevisiae IAM 4428	30	—	0. 62
Sacch. cerevisiae IAM 4702	30	—	
Sp. salmonicolor IFO 1038	30	$+_w$**	
(6) Microalgae (in light)			
Chlamydomonas sp. IAM C-21-1	30	—	3. 36
Scenedesmus basilensis IAM C-66-1	30	—	3. 44
Chlorella vulgaris Al-ly-3	30	—	
Chlorella vulgaris Al-1-3	30	—	3. 38
Chlamydomonas sp. Al-5-2	30	—	3. 47
Chlorella vulgaris Al-16	30	—	3. 90
Chlorella vulgaris Al-28	30	—	3. 90
Scenedesmus obliquus Al-55	30	—	4. 22
(in darkness)			
Chlamydomonas sp. IAM C-21-1	30	—	2. 97
Scenedesmus basilensis IAM C-66-1	30	—	4. 12
Chlorella vulgaris Al-ly-3	30	—	2. 50
Chlorella vulgaris Al-1-3	30	—	3. 52
Chlamydomonas sp. Al-5-2	30	—	5. 34
Chlorella vulgaris Al-16	30	—	5. 46
Chlorella vulgaris Al-28	30	—	3. 12
Scenedesmus obliquus Al-55	30	—	2. 90

* (+) : Growth inhibition was observed, though multiplication in the control medium was poor.

**$+_w$: Weak inhibition

in the sterile *N*/2 NaOH solution.

Sensitivity test of microorganisms to iodinin and its reduced form. Five milliliter of the medium in a test tube was autoclaved at 121. *C* for 15 minutes, cooled, and inoculated with one drop of a 24-hr culture. One tenth milliliter of an alkaline solution of the pigment was added to an inoculated test tube. Fifty microgram or 150 μg of the pig-

ment was added to each culture, and they were all incubated at 30° *C* on a reciprocal shaker, excepting those of lactic acid bacteria and molds, cultivated stationarily. The multiplication was measured by determining the optical density of cultures at 660 *nm* with Hitachi photoelectric photometer type EPO-B. The growth inhibition by iodinin is indicated in percent by the ratio of multiplication in the medium containing the pigment to that in the medium containing no pigment.

Iodinin recovery. After a sensitivity test the pigment in the culture was extracted by chloroform after adjustment of the *pH* value of the culture to less than 3.0 with conc. HCl. The amount of pigment recovered in choroform was determined spectrophotometrically by the method of TANABE and OBAYASHI[5].

RESULTS AND DISCUSSION

Antibiotic action of iodinin

Sensitivities of various kinds of microorganism to growth inhibition by iodinin were determined, as shown in Table 1.

Of bacteria employed for test, members of the genus *Bacillus* except *Bac. cereus* were susceptible to growth inhibition by iodinin at a concentration of less than 5 *μg/ml.* Most of gram-negative bacteria employed for test were unaffected by iodinin at a concentration of 30 *μg/ml.* Minimum inhibitory concencentration of iodinin was determined with iodinin-sensitive strains of *Bacillus.* Some bacteria were found to have changed iodinin into yellow pigments. *Bac. cereus* IFO 3001 and *Ps. azotogena*, both of which were iodinin-insensitive, reduced iodinin into a compound of slight antibiotic action. This compound seemed to be 1,6-phenazinediol, judging from its absorption spectrum in chloroform. The other 4 species of *Pseudomonas* and *E. coli* that are all iodinin-insensitive, are considered to have no ability to reduce iodinin because of high recovery of iodinin.

Most of the lactic acid bacteria employed for test were susceptible to growth inhibition by iodinin, but only two strains of them, *Streptococcus* sp. KK-1-1 and *Lac. plantarum* 11 were unsusceptible to growth inhibition by iodinin, the one was capable of reducing iodinin and the other, incapable.

Most of the actinomycetes employed for test were susceptible to growth inhibition by iodinin. Only one strain of them, *St. ruber* IFO 3110, was unaffected by iodinin.

Many molds employed for test were susceptible to growth inhibition by iodinin. Five species of *Penicillium* employed were completely inhibited by iodinin at a concentration of 10 *μg/ml*; while most strains of *Aspergillus* employed were partly inhibited by iodinin.

Yeasts varied in growth inhibition by iodinin.

Growth of all strains of the microalgae was suppressed for 48 hr in the presence of iodinin at a concentration of 10 *μg/ml*, in light, in the medium containing no glucose, but abundant growth was observed in the subsequent incubation of 5 to 7 days with *Chlorella vulgaris* Al-1-3 and *Chlamydomonas* Al-5-2. In darkness in the medium containing glucose, all strains were not inhibited by iodinin at a concentration of 30 *μg/ml.*

Reaction of microorganisms with 1,6-phenazinediol

The reduced form of iodinin, 1,6-phenazinediol, shows no antibiotic action on most of the bacteria employed for test at a concentration of 30 *μg/ml*, as shown in Table 2. However, 2 strains of them, *Bac. alvei* IFO 3343 and *Bac. circulans* IFO 3329, seemed suppressed with 1,6-phenazinediol. Four strains of the lactic acid bacteria, *Lac. acidophilus* IFO 3831, *Lac. brevis* IFO 3960, *Leuc. mesenteroides* BO7, and *Ped. acidilactici* IFO 3076 were sensitive to 1,6-phenazinediol. The other 3 strains of them, *Lac. plantarum* 11, *Lac. plantarum* K-2, and *Streptococcus* sp. KK-1-1 were insensitive. In the cultures of *Lac. plantarum* 11, *Lac. plantarum* K-2, and *Lac. sake* 012, 1,6-phenazinediol changed into an unknown yellow pigment. The sensitivity of lactic acid bacteria to growth inhibition by 1,6-phenazinediol may be explained by their nutritional complexities. Some species of the actinomycetes were inhibited by 1,6-phenazinediol, but their susceptibility to growth inhibition by it, seems to be fairly lower than that by iodinin. There were many molds which were unaffected by 1.6-phenazinediol. No antibiotic action of 1,6-phenazinediol on growth of yeasts, excepting *Sp. salmonicolor*, at a concentration of 30 *μg/ml* was observed. Growth inhibition of the microalgae by 1,6-phenazinediol at a concentration of 30 *μg/ml* was not observed either in light or in darkness. Growth of the microalgae seemed rather enhanced in the presence of the pigment.

SUMMARY

Iodinin was found to have an inhibitory effect on the growth of many kinds of microorganism (bacteria, actinomycetes, yeasts, molds, and microalgae), varying in their sensitivities. In particular, members of the genus *Bacillus*, excepting *Bac. cereus*, were susceptible to growth inhibition by iodinin. The reduced form of iodinin, 1,6-phenazinediol, showed no antibiotic action on many strains employed in this experiment.

REFERENCES

1. TANABE, I.: *Mem. Fac. Agr. Kagoshima Univ.*, **7**, 55-64 (1969).
2. TANABE, I.: *Mem. Fac. Agr. Kagoshima Univ.*, **8**, 367-372 (1971).
3. McILWAIN, H.: *Nature*, **148**, 628 (1940).
4. McILWAIN, H.: *Nature*, **37**, 265 (1943)
5. TANABE, I. and OBAYASHI, A.,: *Mem.* Fac. Agr. *Kagoshima Univ.*, **8**, 373-389 (1971).
6. IIZUKA, H., TANABE, I., FUKUMURA, T. and KATO, K.: *J. Gen. Appl. Microbiol.*, **13**, 125-137 (1967).
7. PRIDHAM, T. G.: *Antibiotic Annual*, **1956-1957**, 947 (1957).
8. ALEXOPOULOS, CONST., J. and BENEKE, E. S.: *Laboratory Manual for Introductory Mycology*, Burgess Publishing Co., Minn., U. S. A. (1962).