A Screening Method for Antimitotic and Antifungal Substances Using Conidia of *Pyricularia oryzae*, Modification and Application to Tropical Marine Fungi

HISAYOSHI KOBAYASHI, MICHIO NAMIKOSHI[†,*], TAKESHI YOSHIMOTO[†] and TOSHIHIRO YOKOCHI[††]

Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
[†]Department of Ocean Sciences, Tokyo University of Fisheries, Minato-ku, Tokyo 108, Japan
[††]Applied Microbiology Department, National Institute of Bioscience and Human-Technology, Tsukuba, Ibaraki 305, Japan

(Received for publication March 12, 1996)

A bioassay method detecting deformations of mycelia germinated from conidia of *Pyricularia oryzae* P-2b, has been modified to give quantitative estimations. The method was first developed using antimitotic agents which showed characteristic curling effect. Morphological deformations include curling, swelling, hyper-divergency, beads shape and so on, and inhibition of the germination was also observed. For quantitative estimations, indices were introduced for the hyphal growth inhibition and a quantity of conidia in each assay cell and concentration of test solutions were adjusted. Details of the modified method and the application to screening assay of marine fungi isolated in Yap Islands are described. Eight strains of 109 tested showed morphological deformations, and chaetoglobosin A was isolated from the broth filtrate of a strain assigned to *Chaetomium* sp.

This bioassay is a cheap, quick and easy method to be applied to the primary screening for antimitotic and antifungal substances from natural sources.

Morphological deformations observed on mycelia of certain fungi induced by antibiotics and synthetic agents have been known[1~4] and were applied to screening for bioactive secondary metabolites[5~8]. These screening methods detected morphological changes of fungal mycelia grown on agar plates, and test samples were applied by paper disks. However, the methods were limited to a qualitative treatment.

During the research on antimitotic antibiotics, rhizoxins[9], quick and easy bioassay method for antimitotic and antifungal activities has been developed. The antimitotic agents, such as rhizoxin, ansamitocin P-3 and griseofulvin, showed the characteristic curling effect on mycelia of rice plant pathogenic fungus, *Pyricularia oryzae* P-2b, germinated in 0.02% yeast extract solution. The method was found to be useful to detect certain antimitotic compounds by the curling effect and also antifungal compounds by other morphological deformations, such as swelling, hyper-divergency, beads shape and so on, and antifungal antibiotics, fusarielins A~D, were found from the culture broth of a fungus *Fusarium* sp.[10] Although this bioassay method has been applied to the screening for antimitotic and antifungal secondary metabolites from various natural sources, details of the method have not been published.

Since some inquiries of the details have been received, and the method was modified recently, we report here the details of the method including the modification of quantitative estimations. We also mention the application of modified bioassay method to screening from culture broths of marine fungi isolated from Yap Islands and isolation of chaetoglobosin A as the active metabolite of *Chaetomium* sp. Chaetoglobosin A showed weak inhibitory activity to the assembly of microtubule proteins.

Materials and Methods

Preparation of Conidia Suspension

P. oryzae P-2b was grown on a slant culture medium consisted of yeast extract 0.2%, soluble starch 1% and agar 2% at 27°C. The conidia were collected on 12 to 14 days after inoculation by suspending in sterilized water (10 ml). The conidia suspension was filtered to separate from mycelia. The filtrate was added a 2% solution of yeast extract and adjusted to the concentration of 0.02%.

For the quantitative estimation, an aliquot of the conidia suspension was taken on a microscope to count a number of conidia before adding yeast extract. The suspension was adjusted to 4×10^4 conidia/ml by adding sterilized water.

Bioassay

A 96-well flat-bottomed assay plate was used for the bioassay. The first, middle and last columns were pre-

served for negative and positive controls. Rhizoxin was used for positive control with the final concentrations of 1 μM, 0.5 μM, 0.25 μM, 0.125 μM, 60 nM, 30 nM, 16 nM and 8 nM. One column (eight wells) was usually used for one test material with eight different concentrations. The assay plates were incubated at 27°C for 16 hours, and the shape of mycelia germinated from conidia was observed and compared with controls under an inverted microscope.

(1) For water soluble compounds: Water solution of each test material was adjusted the concentration, and 10 μl of the solution was added in an assay well. The conidia suspension (50 μl/well) was then poured into each well and mixed sufficiently. The concentration of conidia is 2×10^3/60 μl/well. Water (10 μl) was added for negative control.

(2) For hydrophobic compounds: Methanol or acetonitrile solution of each test material was added in each well, and the solvent was evaporated in a clean bench. The above conidia suspension was then poured into each well (50 μl/well) and mixed thoroughly (2×10^3 conidia/50 μl/well).

Or the solution of each test material in methanol, acetonitrile or acetone was diluted with water, and 10 μl of the mixture followed by the conidia suspension (50 μl/ml) was added in each well as in the case of water soluble compounds. The final concentration of the solvents was adjusted to less than 5%.

The same procedure was applied without test materials for control together with negative control.

(3) For screening assay: Each 50 μl of conidia suspension was first poured into each well, and 50 μl of each test solution was then added to the first well. If necessary, test materials were first dissolved in organic solvents and diluted with water as in the case of (2), and 50 μl of the solution was added to the first well. The suspension was mixed and taken 50 μl to the second well. The procedure was repeated to the last well of the column. The numbers of conidia were varied well to well as follows: (1) 1×10^3/50 μl/well, (2) 1.5×10^3/50 μl/well, (3) 1.75×10^3/50 μl/well, (4) 1.87×10^3/50 μl/well, (5) 1.94×10^3/50 μl/well, (6) 1.97×10^3/50 μl/well, (7) 1.98×10^3/50 μl/well, (8) 1.99×10^3/50 μl/well.

For negative control, 50 μl of water was added to the first well followed by the procedure as above. If organic solvents were used to dissolve test materials, above procedure with the same solvents was applied for control.

(4) For Tables 1 and 2: Acetone, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), methanol, ethanol or acetonitrile was added to eight wells of one column adjusting the concentration to 10, 8, 6, 4, 2, 1, 0.5 and 0.1% for testing the effect on the hyphal growth of *P. oryzae*. The shape of mycelia and inhibition of conidia germination were observed as above. The results are listed in Table 1.

Fourteen compounds listed in Table 2 were tested as the procedure (2).

Marine Fungi

Fungi used in this study were isolated in a clean room of the research vessel "Sohgen-Maru" during Yap expedition in July 1994 operated by the Marine Biotechnology Institute. Total of 109 different strains were isolated by bating method (56 samples) using wheat, direct smear (14 samples) and by filter-affix (filtered 200 ml of sea water) method (39 samples) utilizing six agar media from seaweeds, marine algae, sea water and marine sediments. The details of isolation will be reported elsewhere.

Isolation of Chaetoglobosin A

Chaetomium sp. was isolated from the green alga, *Halimeda discoidea* by bating method. A small piece (*ca.* 1 cm × 2 cm) of the alga was washed twice with each 2 ml of sterilized 50% sea water containing chloramphenicol (200 μg/ml) in a sterilized test tube and then steeped in the same solution (4 ml) for 24 hours in a sterilized plate. Two pieces of sterilized wheat were added to the solution after removal of the alga. The plate was incubated at 25°C for 3 days, and mycelia grown on the wheat were taken to an agar plate (glucose 2%, polypeptone 1%, yeast extract 0.5%, chloramphenicol 200 μg/ml, penicillin G 50 μg/ml and agar 2% in 90% sea water, pH 6.5). The agar plate was incubated at 25°C, and the mycelium grown to the edge of plate was taken to a slant of potato dextrose agar (a half nutrient, 50% sea water).

The fungus was inoculated into 10 plates of potato dextrose medium (each 25 ml, a half nutrient in 50% sea water), and the plates were incubated at 20 °C for 3 weeks. The broth was filtered, and the filtrate (*ca.* 200 ml) was passed through an HP-20 column (40 ml). The column was washed with water (100 ml) and eluted with methanol (130 ml). The residue after evaporation of methanol was partitioned with water (40 ml), acetone (30 ml) and benzene (30 ml). The organic layer was concentrated, and the residue (40 mg) was chromatographed on silica gel (5 g) with benzene, benzene-acetone and then methanol. The benzene - acetone (5 : 1) fraction showed the activity in the bioassay and was concentrated to give 7 mg of chaetoglobosin A.

The quantitative analysis of chaetoglobosin A in the broth filtrate was performed by HPLC with an ODS column (6 mm × 15 cm), acetonitrile - water (55 : 45) as solvent (1.0 ml/minute) and a UV detector at 254 nm (equipped with a data processor). A calibration table was created by injecting the standard solution of pure chaetoglobosin A. The calibration curve was calculated by linear-least squares regression. The broth filtrate (10 μl) was injected and the chromatogram was integrated in terms of concentration. The procedure was in triplicate to demonstrate that 61.8 μg of chaetoglobosin A were contained in 1 ml of the broth filtrate.

Spectral Analysis

NMR spectra were measured on a JEOL JNM A-500 NMR spectrometer in $CDCl_3$ and TMS as internal

standard. Mass spectra were obtained by a JEOL HX-110 mass spectrometer. UV and IR spectra were recorded on a Shimadzu UV-300 and on a JASCO A-102, respectively.

Preparation of Microtubule Protein and Microtubule Assembly Assay were performed as described previously[10].

Antifungal Activity

Antifungal activities of cytochalasans listed in Table 4 were tested by the dilution method.

Results and Discussion

Details of Bioassay

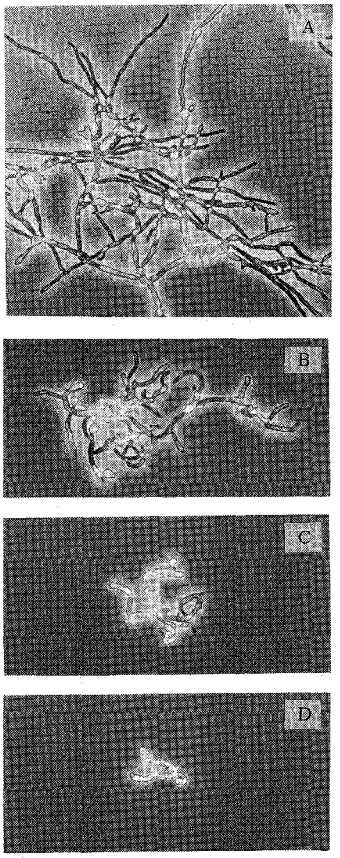
Conidia of *P. oryzae* P-2b germinated at 27°C in water suspension containing 0.02% yeast extract and were easily observed under an optical microscope. After 16 hours of incubation, hyphal growth was the most appropriate for observation (Fig. 1A). Rhizoxin caused the characteristic curling effect on mycelia as shown in Fig. 1.

The effect of six water miscible organic solvents, acetone, DMSO, DMF, ethanol, methanol and acetonitrile, on the hyphal growth of *P. oryzae* was examined (Table 1), since hydrophobic compounds and extracts would be first dissolved in such organic solvents and then evaporated the solvents or diluted with water for bioassay. DMSO, DMF and ethanol inhibited the hyphal growth at rather lower concentration (Table 1). Acetonitrile was found to be the most suitable solvent, and acetone and methanol are also usable at the concentration of less than 5%. It should be noted that acetone damages the plastic assay plates. The test materials were dissolved, if necessary, in acetonitrile or methanol and then either evaporated the solvent in assay wells or diluted with water (acetone may be used for this purpose).

The bioassay system was then examined with antitumor and antifungal agents to evaluate the applicability (Table 2). The antimitotic antibiotics, rhizoxin, ansamitocin P-3 and griseofulvin, showed the characteristic curling effect. The first two compounds strongly inhibited the hyphal growth with curling effect even at lower concentration. Similar effects were observed for thiabendazole and nocodazole, synthetic antifungal agents with antimitotic activity. Other antimitotic agents, vinblastine, vincristine and colchicine, showed weak curling effect at higher concentration (100, 100 and 50 μg/ml, respectively). The morphological deformation by these

Fig. 1. Curling effect on mycelia germinated from conidia of *Pyricularia oryzae* P-2b induced by rhizoxin.

A: control (water); index, −. B: rhizoxin 10 ng/ml (16 nM); index, +. C: rhizoxin 20 ng/ml (32 nM); index, 2+. D: rhizoxin 50 ng/ml (80 nM); index, 3+.



Conidia were incubated in sterilized water containing 0.02% yeast extract with or without rhizoxin.

Table 1. Effect of organic solvents on mycelia of *Pyricularia oryzae* P-2b.

Organic solvent	Concentration (%)						
	10	8	6	4	2	1	0.5
Acetone	+	+	−	−	−	−	−
Dimethyl sulfoxide	×	×	+	−	−	−	−
Dimethylformamide	×	×	×	×	+	−	−
Ethanol	×	2+	+	+	−	−	−
Methanol	+	+	−	−	−	−	−
Acetonitrile	+	−	−	−	−	−	−

2+, +, −, ×: See text.

Table 2. Effect of antitumor and antifungal agents on *Pyricularia oryzae* P-2b.

Compound	Curling effect[a]	Hyphal growth inhibition[b] (μg/ml)		
		50	5	0.5
Rhizoxin	A	3+	3+	3+
Ansamitocin P-3	A	3+	3+	3+
Griseofulvin	A	+	±	−
Vinblastine sulfate	M	−	−	−
Vincristine sulfate	M	±	−	−
Colchicine	M	−	−	−
Thiabendazole	A	+	−	−
Nocodazole	A	3+	+	−
Taxol	W	+	−	−
Amphotericin B	N	×	×	+
Econazole	N	×	+	−
Kasugamycin	N	×	2+	−
Cycloheximide	N	×	×	×
5-Fluorocytosine	N	×	+	−

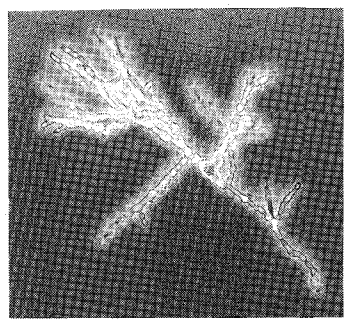
[a] A: Apparent; M: marginal; W: weak; N: no deformation.
[b] 3+, 2+, +, ±, −, −: See text.

compounds was difficult to detect as a screening method. Taxol, the antimitotic agent with assembly promotion and disassembly inhibition activities to microtubule, revealed weak curling effect with growth inhibition at 50 μg/ml. The antifungal agents, amphotericin B and econazole (disrupt fungal cell membrane), kasugamycin and cycloheximide (inhibit protein synthesis) and 5-fluorocytosine (inhibits DNA synthesis) inhibited the conidia germination and hyphal growth but showed no morphological deformation. Thus, the bioassay method was found to be useful to detect certain antimitotic compounds by the characteristic curling effect of mycelia and antifungal agents by morphological deformations and/or growth inhibition of mycelia.

Quantitative Estimation

A bioassay detecting morphological changes on organisms is not usually applicable to quantitative treatment, and the method described in this study was neither suitable for that purpose at the beginning. The method, however, has recently been modified to give quantitative estimations. The indices of hyphal growth inhibition used for quantitative estimations were divided into six classes (−, ±, +, 2+, 3+ and ×) according to the length of mycelia germinated from conidia. The deformations of mycelia were indicated by the shape observed, such as curling, swelling, hyper-divergency, beads shape and so on, with the above symbols. The symbol "−" shows no effect, that is, the same as negative control (Fig. 1A). The magnitude of hyphal growth inhibition indicated by 3+, 2+ and + is shown in Fig. 1B~D with curling effect induced by rhizoxin. When mycelia showed a morphological deformation but no growth inhibition of mycelia, the symbol "±" was used with the type of deformation.

Fig. 2. Beads shape deformation induced by chaetoglobosin A (3.5 μg/ml).



The inhibition of conidia germination (symbol ×) was scored by 100% inhibitory concentration (IC_{100}). This index would be more precise than that used for morphological deformations since detection of the inhibitory activity of conidia germination is quite objective. Although IC_{50} could also be determined, IC_{100} should be more accurate to use for the index.

A number of conidia in each well was adjusted to extend quantitative estimations. Thus, the index of morphological deformations can be compared directly among different materials by the concentration.

Application to Screening

The application of this bioassay to the screening of bioactive secondary metabolites from various natural sources has easily been accomplished. Activities of extracts can be compared directly if the concentration of solution in each assay well is adjusted. For culture broths of microorganisms, comparison of activity would be made at the same dilution, which are usually at the same row of an assay plate.

The modified bioassay method was applied to the screening for antimitotic and antifungal secondary metabolites of fungi isolated from reef of Yap Islands. Mycelial extracts and broth filtrates of 109 stains were tested the activity, and eight strains showed morphological deformations, curling (+), beads (three strains, each 2+), swelling (2+) and hypertrophy (three strains, each +). Broth filtrates of four strains inhibited conidia germination at rather lower concentrations. The isolation of compound responsible to the activity has first been

made from the strain assigned to *Chaetomium* sp., which showed the strongest and characteristic activity (beads, Fig. 2) among these fungi.

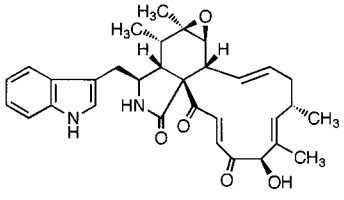
The bioassay guided separation as described in the Materials and Methods section gave 7 mg of active compound from 250 ml of the culture broth. The structure was elucidated based on the spectral data, such as ^{1}H and ^{13}C NMR, mass, IR and UV spectra, and assigned to chaetoglobosin A[11] (Fig. 3). The assignment was confirmed by the direct comparison with the authentic sample.

Chaetoglobosin A and Cytochalasans

Chaetoglobosin A showed beads shape deformation (Fig. 2) on mycelia of *P. oryzae* at the concentration as low as 0.5 μg/ml. This morphological deformation has not been observed with antifungal agents tested and was first detected with this compound. The microtubule assembly assay was performed according to the method described by SHELANSKI *et al.*[10,12], and chaetoglobosin A showed weak inhibitory activity (Fig. 4) as identical to the result reported by SATO *et al.*[13].

Since the effect of cytochalasans, which affect to microfilaments, on hyphal growth of *P. oryzae* was an interesting question, 12 compounds listed in Table 3 were tested the activity. Among the 12 compounds, only chaetoglobosin A showed the beads shape deformation, and chaetoglobosin J inhibited conidia germination at 5 μg/ml and weak hypertrophy of mycelia with growth inhibition at 0.25 μg/ml. Aspochalasins B and D showed similar but weaker activities to those of chaetoglobosin J. Other compounds were inactive at the concentration of 50 μg/ml. The antifungal activities of these compounds to phytopathogenic fungi revealed that only chaetoglobosin A was active to three of 10 fungi at the concentration of less than 50 μg/ml (Table 4). Chaetoglobosin A also showed acute toxicity[14] and teratogenic activity[15] to mice. These activities would, therefore, be characteristic to only chaetoglobosin A among the cytochalasans.

Fig. 3. Structure of chaetoglobosin A.



Conclusion

Application of *in vitro* biochemical reactions, such as enzyme activities, is, recently, the leading for screening methods, so called targeted screening. While the detection of morphological deformations observed on living

Fig. 4. Inhibitory activity of chaetoglobosin A and rhizoxin to microtubule assembly.

○ Control, △ chaetoglobosin A 50 μM, ▲ chaetoglobosin A 100 μM, ■ rhizoxin 10 μM.

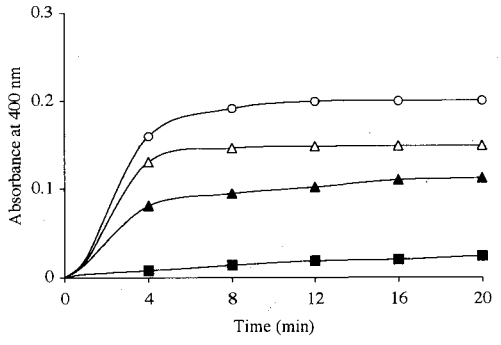


Table 3. Effect of cytochalasans on mycelia of *Pyricularia oryzae* P-2b.

Compound	50	10	5	1 (μg/ml)	0.5	0.25	0.125
Chaetoglobosin A	×	3+[a]	2+[a]	+[a]	+[a]	−	−
Chaetoglobosin B	−	−	−	−	−	−	−
Chaetoglobosin C	−	−	−	−	−	−	−
Chaetoglobosin D	−	−	−	−	−	−	−
Chaetoglobosin E	−	−	−	−	−	−	−
Chaetoglobosin F	−	−	−	−	−	−	−
Chaetoglobosin J	×	×	×	+[b]	+[b]	+[b]	−
Aspochalasin B	×	3+[b]	−	−	−	−	−
Aspochalasin D	×	−	−	−	−	−	−
Cytochalasin B	−	−	−	−	−	−	−
Cytochalasin H	−	−	−	−	−	−	−
Epoxycytochalasin H	−	−	−	−	−	−	−

[a] Beads shape deformation. [b] Weak hypertrophic deformation.
3+, 2+, +, −, ×: See text.

Table 4. Antifungal activity of cytochalasans to phytopathogenic fungi.

Compound	MIC (μg/ml)									
	1	2	3	4	5	6	7	8	9	10
Chaetoglobosin A	–[a]	25	13	–	–	–	25	–	–	–
Chaetoglobosin B	–	–	–	–	–	–	–	–	–	–
Chaetoglobosin C	–	–	–	–	–	–	–	–	–	–
Chaetoglobosin D	–	–	–	–	–	–	–	–	–	–
Chaetoglobosin E	–	–	–	–	–	–	–	–	–	–
Chaetoglobosin F	–	–	–	–	–	–	–	–	–	–
Chaetoglobosin J	–	–	–	–	–	–	–	–	–	–
Aspochalasin B	–	–	–	–	–	–	–	–	–	–
Aspochalasin D	–	n[b]	n	–	n	–	n	–	–	n
Cytochalasin H	–	–	–	–	–	–	–	–	–	–
Epoxycytochalasin H	–	–	–	–	–	–	–	–	–	–
(+)-Sclerotiorin	–	–	–	–	–	–	–	–	–	–
(+)-Isorotiorin	–	–	–	–	–	–	–	–	–	–

1. *Alternaria kikuchiana*. 2. *Colletotricum lindemuthianum*. 3. *Fusarium nivale*. 4. *Fusarium oxysporum* f. sp. *lycopersici*. 5. *Fusarium solani* f. sp. *phaseoli*. 6. *Helminthosporium oryzae*. 7. *Pythium aphanidermatum*. 8. *Pyricularia oryzae*. 9. *Rhizoctonia solani*. 10. *Rhizopus chinensis*.

[a] –: ≥50 μg/ml. [b] n: Not tested.

organisms seems to be somewhat inefficient, since it depends on subjective viewpoints of observers and is less quantitative. However, such observation of reactions detected on organism would, in some cases, leads to unexpected discoveries as Beppu pointed out in his review[5]. It could be dependent upon careful and keen observations by researchers.

From the above point of view, the bioassay method described in this study would be useful for applying to the screening for antimitotic and antifungal activities of secondary metabolites from various natural sources and is quick, easy and cheap method. Since this bioassay observes morphological deformations of mycelia right on the germination from conidia, quantitative estimations of the results can be applicable compared to other methods which detect morphological changes.

The separation of metabolites responsible to morphological deformations of mycelia of *P. oryzae* from other active culture broths of marine fungi is now in progress.

Acknowledgments

This study was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan to MN. We thank Drs. S. MIYACHI and M. ENDO of Marine Biotechnology Institute, Inc. for our participation to the Yap expedition by "Sohgen-Maru", Dr. N. MORISAKI for mass spectra and Ms. Y. KOISO for the microtubule assembly assay. We also thank Professor SHIGEO IWASAKI for useful discussion and extensive support throughout the work and would like to dedicate this paper to him on the occasion of his 60th birthday on 21st of June.

References

1) RICHMOND, D. V.: Effects of toxicants on the morphology and fine structure of fungi. Adv. Appl. Microbiol. 19: 289~319, 1975
2) BRIAN, P. W.: Studies on the biological activity of griseofulvin. Ann. Bot. 13: 59~77, 1949
3) BETINA, V. & D. MICEKOVA: Morphogenetic activity of cytochalasins, cyanein, and monorden in *Botrytis cinerea*. Z. Allg. Mikrobiol. 13: 287~298, 1973
4) ISONO, K.; K. KOBINATA & S. SUZUKI: Isolation and characterization of polyoxins J, K and L, New components of polyoxin complex. Agric. Biol. Chem. 32: 792~793, 1968
5) BEPPU, T.: Morphological abnormalities of eukaryotic cells induced by microbial metabolites as indices to direct nobel physiological activities. Protein, Nucleic Acid and Enzyme 38: 1639~1646, 1993
6) GUNJI, S.; K. ARIMA & T. BEPPU: Screening of antifungal antibiotics according to activities inducing morphological abnormalities. Agric. Biol. Chem. 47: 2061~2069, 1983
7) MARUMO, S.; M. NUKINA, S. KONDO & K. TOMIYAMA: Lunatoic acid A a morphogenic substance inducing chlamydospore-like cells in some fungi. Agric. Biol. Chem. 46: 2399~2401, 1982
8) SAKAMOTO, K.; S. IZUMI, M. MIYACHI & M. OKUHARA: A new assay method for immunosuppressants with a tacrolims (FK 506)-like mode of action. J. Antibiotics 48: 727~729, 1995
9) TAKAHASHI, M.; S. IWASAKI, H. KOBAYASHI, S. OKUDA, T. MURAI, Y. SATO, T. HARAGUCHI-HIRAOKA & H. NAGANO: Studies on macrocyclic lactone antibiotics XI. Antimitotic and anti-tubulin activity of new antitumor antibiotics, rhizoxins and its homologues. J. Antibiotics 40: 66~72, 1987
10) KOBAYASHI, H.; R. SUNAGA, K. FURIHATA, N. MORISAKI & S. IWASAKI: Isolation and structures of an antifungal antibiotic, fusarielin A, and related compounds produced by a *Fusarium* sp. J. Antibiotics 48: 42~52, 1995
11) SEKITA, S.; K. YOSHIHIRA, S. NATORI & H. KUWANO: Structures of chaetoglobosins A and B, cytotoxic metabolites of *Chaetomium globosum*. Tetrahedron Lett. 1973:

2109～2112, 1973

12) SHELANSKI, M.; F. GASKIN & C. R. CAUTER: Microtubule assembly in the absence of added nucleotides. Pro. Natl. Acad. Sci. U.S.A. 70: 765～768, 1973

13) SATO, Y.; Y. SAITO, T. TEZUKA, S. SEKITA, K. YOSHIHARA & S. NATORI: Viscometric analysis of effects of cytochalasans on *in vitro* polymerization and depolymerization of microtubules. J. Phar. Dyn. 5: 418～422, 1982

14) UMEDA, M.; K. OHTSUBO, M. SAITO, S. SEKITA, K. YOSHIHIRA, S. NATORI, S. UDAGAWA, F. SAKABE & H. KURATA: Cytotoxicity of new cytochalasans from *Chaetomium globosum*. Experientia 31: 435～439, 1975

15) SEKITA, S.; K. YOSHIHIRA, S. NATORI, S. UDAGAWA, F. SAKABE, H. KURATA & M. UMEDA: Chaetoglobosins, cytotoxic 10-(indol-3-yl)-[13]cytochalasans from *Chaetomium* spp. Chem. Pharm. Bull. 30: 1609～1617, 1982