A SCREENING METHOD FOR ANTIFUNGAL SUBSTANCES USING *SACCHAROMYCES CEREVISIAE* STRAINS RESISTANT TO POLYENE MACROLIDES

GILLES ETIENNE, ELISE ARMAU† and GÉRARD TIRABY

Laboratoire de Microbiologie et Génétique appliquées du C.N.R.S., Université Paul Sabatier, 118 route de Narbonne, 31062 TOULOUSE Cédex, France
†Laboratoire de recherche CAYLA, Avenue de Larrieu, 31094 TOULOUSE Cédex, France

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Strains of *Saccharomyces cerevisiae* FL200 capable of growing on a solid medium containing a mixture of polyene macrolide antibiotics (nystatin, 40 μg/ml, amphotericin B, 40 μg/ml, pimaricin, 150 μg/ml and RP9971 antibiotic, 10 μg/ml) have been isolated after successive selection steps. The mutant strains, PR13 and PRC24, are 10 to 100 times more resistant than the wild-type strain to 8 polyene macrolide antibiotics. When 4% Tween 80 is added to the medium, resistance to these antifungal drugs is further increased. In addition, strain PRC24, derived from strain PR13, is resistant to a non-polyene macrolide antifungal antibiotic, cycloheximide. In contrast, PR13 and PRC24 are both highly susceptible to a large range of compounds, including non-polyenic antifungal, antitumor and antibacterial agents. These particular characteristics make these strains useful for the rapid detection of antifungal compounds of the polyene macrolide and cycloheximide types, as well as for the recognition of antimitotic substances.

Since the discovery of the first polyene macrolide antibiotics in the early 1950's, more than 100 different compounds belonging to this family have been described[1], and more are being discovered every year. However only those compounds isolated in the early period are used in human therapy, as none of those discovered since then have been shown to be superior in clinical trials. The primary reason is that polyene macrolides display a relatively high toxicity for human even though their inhibitory potency against most pathogenic fungi and yeasts is rather high. In a program to screen for bacteria synthesizing new antifungal substances distinct from the polyene macrolides, it is necessary to avoid the polyene macrolide producers. Since polyene macrolides are produced by actinomycetes, particularly *Streptomyces*, where as many as 34 to 88% of fresh isolates have been reported to be producers, it is of prime importance to identify producing strains as early as possible. Positive strains are discarded with the exception of those isolates which produce additional antifungal activities.

Polyenes are easily characterized by a typical UV absorption spectrum due to the presence of conjugated double bonds in the macrolactone ring of the molecule[1]. However, this spectrophotometric identification requires a butanol extraction, usually of liquid cultures, and therefore limits the number of strains that can be handled. In contrast microbiological methods allow the analysis of a greater number of strains at an early stage of the screening procedure. Some differential assays to recognize polyene macrolides can be inferred from the literature; sterols, and particularly ergosterol[2], antagonize the antifungal activity of these compounds[3,4]. Similarly, it has been reported that fatty acids[5] or Tween 80[6] also interfere with the biological activity of polyene macrolides. On the other hand, there have been many reports of the isolation of *Saccharomyces cerevisiae* mutants selectively resistant to polyene macrolides[7~9]. We have combined these different methods to elaborate a screening procedure selective to the polyene macrolides.

The screen is based on the use of *S. cerevisiae* mutants completely insensitive to these antibiotics in presence of 4% Tween 80. The polyene macrolide-resistant mutants obtained through successive selection steps turned out to be highly sensitive to many non-polyene macrolide antifungal substances, including cycloheximide, an antibiotic frequently encountered in microbial culture media[10]. From the highest polyene macrolide-resistant mutant of *S. cerevisiae*, a cycloheximide-resistant strain has been isolated, which allows an early elimination of both of these families of antifungal compounds.

Materials and Methods

Antifungal Antibiotics

Filipin (The Upjohn Company, Kalamazoo, Michigan, U.S.A.), rimocidin (Pfizer International Inc., Groton, Connecticut, U.S.A.), nystatin, RP7071 and RP9971 (Rhône-Poulenc, Paris, France) were gifts from the corresponding company. Amphotericin B (Squibb Laboratories, Neuilly sur Seine, France), candicidin (Calbiochem, San Diego, California, U.S.A.), cycloheximide (Sigma Chemicals, St Louis, Missouri, U.S.A.) and pimaricin (Gist-Brocades, Delft, Netherlands) were obtained from commercial sources.

Stock solutions of polyene macrolides were 10 mg/ml in 10% dimethyl sulfoxide.

Mutagenesis

The *S. cerevisiae* FL200 wild type strain provided by Dr. F. LACROUTE (Gif-sur-Yvette, France) was used as the parental strain. The mutant strains isolated from FL200 were selected after mutagenesis of exponentially growing cells in YPG broth (yeast extract (Difco) 1%, Neopeptone (Difco) 1% and glucose 2%). Mutagenesis was performed with nitrous acid (3 mM in 0.05 M acetate buffer, pH 4.0) for 30 minutes to give 1% survival. The mutagenized cells were plated on YPG agar containing polyene macrolide antibiotics at lethal concentrations. The cells were incubated for 5 days at 32°C.

Polyene Susceptibility Assay

MICs were determined by a 3-fold serial dilution procedure onto YPG agar plates. A diluted cell suspension of each strain was spotted on the agar plates; inoculum size was about 10^5 cfu/ml. The yeast cells were prepared by suspending a 2 day-old *S. cerevisiae* culture grown on YPG agar in 10 ml of sterile YPG broth. MICs were recorded after a 2 days incubation at 32°C.

Susceptibility Disk Assay Method

Just before use, 150 ml of YPG agar were inoculated with 0.2 ml of yeast suspension prepared as previously described; when indicated, Tween 80 (E. Merck, Darmstadt, Germany) or ergosterol (Aldrich-Europe Div., Beerse, Belgium) solubilized in 95% ethanol were added aseptically to the medium, at a final concentration of 4% and 300 μg/ml, respectively. The medium was then gently mixed and poured into sterile plates (Nunc Inter-Med, 245 × 245 mm dishes for diffusion tests). 10 μl of the stock solution of each antibiotic were adsorbed onto 10 mm paper disks (Whatman No. 1) and the disks were placed in duplicate on the surface of the yeast seeded agar plates. The plates were held for 2 hours at 4°C in order to facilitate diffusion of the antibiotics and the plates were then incubated overnight at 32°C.

Results

Inactivation of Polyene Antibiotics by Tween 80 and Ergosterol

The antagonistic effects of Tween 80 and ergosterol against eight polyene macrolides, representative of the major subgroups of this family of antibiotics (*i.e.* tetraenes, pentaenes and heptaenes) have been compared (Table 1). Both compounds interfere with the antifungal activity of the polyene macrolides, as demonstrated by the decreased size of the inhibition zone around disks containing antifungal compounds

on strain FL200 growing in the presence of these chemicals. However, Tween 80 shows a more pronounced effect than ergosterol on the activity of the different polyene macrolides with the exception of compounds RP7071 and RP9971. The combination of both ergosterol and Tween 80 has failed to confer a more pronounced inactivation of the polyene macrolides than does Tween 80 alone, except with candicidin and filipin which are completely inactivated. Moreover, with these conditions, the growth rate of wild-type *S. cerevisiae* is drastically reduced, when compared to growth rate when either Tween 80 or ergosterol are added alone to the culture medium. Considering the efficiency of 4% Tween 80 in decreasing the antifungal activity of polyene macrolides, we finally decided to use this surfactant alone in order to screen selectively against these antifungal drugs.

The anionic detergent, Tween 80, could interfere with the antimicrobial activity of some antibiotics; consequently, the effect of Tween 80 upon the antifungal activity of compounds which are representative of the major class of antibiotics that inhibit the growth of wild-type *S. cerevisiae* (*e.g.* non-polyene macrolide antifungal, antimitotic, ionophorous and aminoglycosidic antibiotics) has been determined (Table 2). The

Table 1. Effect of ergosterol and Tween 80 upon the activity of polyene macrolide antibiotics.

Polyenes	Inhibition zone diameter (mm)			
	YPG agar added with			
	No addition	Tween 80 4%	Ergosterol 300 μg/ml	Tween 80 4% + ergosterol 300 μg/ml
Tetraenes				
Nystatin	28	18	22.5	15
Pimaricin	28.5	21	24.5	22
Rimocidin	28.5	18	21	16
RP7071	27	17.5	14.5	16.5
RP9971	28	18	18	17
Pentaene				
Filipin	21	10.5	15	—
Heptaenes				
Amphotericin B	12.5	—	—	—
Candicidin	18	12.5	13.5	—

Each antibiotic was tested in duplicate on wild-type *Saccharomyces cerevisiae* FL200 using 10 mm paper disks with 100 μg antibiotic per disk.
—: No inhibition zone around the disk.

Table 2. Effect of Tween 80 upon the activity of some antibiotics exhibiting an antifungal activity.

	Inhibition zone diameter (mm)			Inhibition zone diameter (mm)	
	YPG agar	YPG 80 agar[a]		YPG agar	YPG 80 agar[a]
Flucytosine[b]	(22.5)	(20)	Clotrimazole[c]	20	—
Lomofungin[b]	14	11.5	Bleomycin[c]	26	25
Thiolutin[b]	30.5	23.5	G418[c]	22	21
Paromomycin[b]	19	19	Hygromycin B[c]	18	17
Polymyxin B[b]	19	18.5	Streptothricin D[c]	19	19
Cycloheximide[c]	18.5	18.5			

Numbers in parenthesis indicate a cloudy inhibition zone.
Each antibiotic was tested in duplicate on wild-type *Saccharomyces cerevisiae* FL200 using 10 mm paper disks:
[a] YPG 80: YPG medium added with 4% Tween 80. [b] 100 μg antibiotic per disk. [c] 10 μg antibiotic per disk.
—: No inhibition zone around the disk.

majority of these antibiotics were found to exhibit similar activities whether or not 4% Tween 80 was added to the medium. In contrast, thiolutin, lomofungin and particularly clotrimazole behave like the polyene macrolide antibiotics; therefore utilization of Tween 80 alone to selectively characterize the polyene macrolides is not adequate, since other antifungal drugs may also be antagonized by this compound.

Isolation and Description of Mutants

Strains of *S. cerevisiae* which are not susceptible to the polyene macrolides have been isolated by successive selection of mutants resistant to increasing concentrations of antifungal drugs; in order to obtain a wide range of cross-resistance to various polyene macrolides, a different polyene macrolide was used for each selection step.

Following mutagenesis, wild-type *S. cerevisiae* FL200 was subjected to a mixture of nystatin and amphotericin B, representing two subgroups of the polyene macrolides family. Subsequent rounds of selection led to the isolation of spontaneous pimaricin-resistant mutants, including strain P150b and later

Fig. 1. Isolation of mutant strains of *Saccharomyces cerevisiae* highly resistant to the polyene macrolide antibiotics.

FL200 (wild-type strain)
| HNO_2 mutagenesis
NAP40b (isolated on YPG medium plus nystatin and amphotericin B (40 μg/ml each))
| spontaneous mutation
P150b (isolated on YPG medium plus pimaricin (150 μg/ml) added with amphotericin B and nystatin (40 μg/ml each))
| HNO_2 mutagenesis
PR13 (isolated on YPG medium plus RP7071 (3 μg/ml) added with pimaricin (150 μg/ml) and amphotericin B and nystatin (40 μg/ml each))
| HNO_2 mutagenesis
PRC24 (isolated on YPG medium plus cycloheximide (3 μg/ml) added with pimaricin and nystatin (40 μg/ml each))

Table 3. Susceptibility of the wild-type and mutant strains of *Saccharomyces cerevisiae* towards polyene macrolides and cycloheximide.

Antibiotics	MICs (μg/ml)				
	Wild-type FL200	Resistant mutants			
		NAP40b	P150b	PR13	PRC24
Tetraenes					
Nystatin	30	100	300	1,000	1,000
Pimaricin	10	30	100	1,000	1,000
Rimocidin	3	10	30	100	100
RP7071	0.3	1	3	10	10
RP9971	0.3	1	3	10	10
Pentaene					
Filipin	100	100	100	100	100
Heptaenes					
Amphotericin B	10	100	100	100	100
Candicidin	10	30	30	300	300
Cycloheximide	30	0.3	0.3	0.3	100

to the isolation of strain PR13 which is resistant to RP7071 (Fig. 1).

After only one selection step, the polyene macrolide-resistant strains were found to be 100 times more susceptible to cycloheximide than the wild-type strain FL200 (Table 3). An additional round of mutagenesis and selection of strain PR13, produced a cycloheximide-resistant mutant, PRC24.

Table 3 records the resistance levels of strains isolated from the successive selection steps. Only filipin was found to have an identical MIC for the wild-type and the mutant strains. For the other polyene macrolides, all the mutant strains displayed identical patterns of cross-resistance: With the exception of amphotericin B, the MICs increase continuously from strain NAP40b to strain PR13. Strain PRC24, which has maintained the polyene macrolide-resistance trait from strain PR13 is slightly more resistant to cycloheximide than the wild-type strain.

Detection of Antifungal Antibiotics: Combination of Tween 80 with the Mutant Strain PRC24

The susceptibility disk assay method was used in all of the following studies because diffusion of polyene macrolides is poor, due to their relative hydrophobic properties, in agar media; thus, there is not necessarily a correlation between MICs and the zones of inhibition. On the other hand, we could expect that results obtained from the susceptibility disk assay method could be directly extended to those obtained by the agar plug method used to screen actinomycetes strains for antifungal activities.

We have combined the use of the mutant strain PRC24 and Tween 80 to look for complete resistance to the polyene macrolide antibiotics. In the presence of 4% Tween 80, strain PRC24 is completely resistant to all the polyene macrolides tested (Table 4). In addition, this strain exhibits an increased susceptibility to the non-polyenic antibiotics. The fact that 4% Tween 80 interfered with the thiolutin and lomofungin activities (Table 2) is overcome by the increased susceptibility of the mutant strain PRC24 to these compounds.

It was noted that clotrimazole, a synthetic imidazole derivative, is indistinguishable from polyene macrolides with respect to the Tween 80 antagonistic effect and the resistance of the PRC24 strain

Table 4. Susceptibility of strains of *Saccharomyces cerevisiae* to antibiotics exhibiting antifungal activities.

Antibiotics	Inhibition zone diameter (mm)		Antibiotics	Inhibition zone diameter (mm)	
	Strain FL200 (YPG agar)	Strain PRC24 (YPG 80 agar)		Strain FL200 (YPG agar)	Strain PRC24 (YPG 80 agar)
Polyene macrolides			Lomofungin[a]	14	15.5
Amphotericin B[a]	12	—	Paromomycin[a]	16.5	21.5
Candicidin[a]	19.5	—	Polymyxin B[a]	19	20
Filipin[a]	21.5	—	Cycloheximide[b]	18.5	—
Nystatin[a]	25	—	Clotrimazole[b]	20	—
Pimaricin[a]	24	—	Bleomycin[b]	26	34
Rimocidin[a]	26	—	G 418[b]	22	31.5
RP7071[a]	27	—	Hygromycin B[b]	18	30.5
RP9971[a]	26.5	—	Streptothricin D[b]	19	26.5
Other antibiotics			Thiolutin[b]	15.5	26
Flucytosine[a]	22.5	22			

Each antibiotic was tested in duplicate using 10 mm paper disks: [a] 100 μg antibiotic per disk. [b] 10 μg antibiotic per disk.

—: No inhibition zone around the disk.

Table 5. Susceptibility of the strain PRC24 to various antibiotics.

Antibiotics	Strain FL200 (YPG agar)	Strain PRC24 (YPG 80 agar)	Antibiotics	Strain FL200 (YPG agar)	Strain PRC24 (YPG 80 agar)
Antibacterials[a]			Monensin	—	19
Apramycin	—	18	Nigericin	—	20.5
Neomycin	—	14	Colistin	—	17.5
Butirosin	—	—	Novobiocin	—	14.5
Kanamycin	—	18	Antimitotics[b]		
Tobramycin	—	15	Actinomycin D	—	21
Gentamicin	—	21	Doxorubicin	—	25
Fortimicin A	—	—	Daunorubicin	—	27
Tetracycline	—	11	Echinomycin	—	21
Minocycline	—	17	Mitomycin	—	—
Erythromycin	—	12.5	Nogalamycin	—	20
Spiramycin	—	—	Geldanamycin	—	11.5

[a,b] See Table 4.

to this compound.

Because of this increased susceptibility of strain PRC24 to some antifungal compounds, we have tested the susceptibility of this mutant to various antitumor and antibacterial antibiotics which are inactive on the wild-type strain FL200. Strain PRC24 was found to be susceptible to various antibacterial antibiotics (Table 5); many of these products (aminoglycosides, tetracyclines, macrolides) are known to act mainly on the 70S ribosomes to inhibit protein synthesis. Antibiotics acting on DNA replication (novobiocin) or on the cell membrane (monensin, nigericin, colistin and polymyxin B) also interfere with the growth of strain PRC24. As expected, antibiotics which inhibit the synthesis of the bacterial cell wall (β-lactams, bacitracin, phosphonomycin) or block prokaryotic RNA polymerase (rifampicin) are completely inactive on mutant strain PRC24 (data not shown).

Antimitotic antibiotics of microbial origin (except mitomycin) are remarkably efficient in inhibiting growth of strain PRC24 (Table 5). In contrast, all of the 22 different antitumor compounds of non-microbial origin (*e.g.* cytarabine, fluorouracil, hydroxyurea, ifosfamide, vincristin) which are used in chemotherapy in France fail to inhibit strain PRC24 (data not shown).

Almost all of the antibacterial and antitumor antibiotics tested give identical inhibition zones on mutant strain PRC24 with or without 4% Tween 80 except for geldanamycin, doxorubicin and daunorubicin (data not shown). As for thiolutin and lomofungin, the slight antagonistic effect of Tween 80 upon the activity of these products is overcome by the increased susceptibility of strain PRC24.

Evaluation of the Screening Method

Twelve ATCC strains which are producers of antifungal compounds were tested in the screening system (Table 6). The 6 strains which produce polyene macrolide antibiotics were either inactive, or much less active, upon the mutant strains PR13 and PRC24 (YPG 80 agar) than upon the wild-type strain of *S. cerevisiae* FL200; the weak activity of *Streptomyces rimosus* towards the mutant strains can be ascribed to tetracycline, which is coproduced with rimocidin by *S. rimosus*. *Streptomyces naraensis*, a cycloheximide producing strain, also showed a typical spectrum of action, as it was highly active on the mutant strain PR13, and much less active upon FL200 and especially upon PRC24. On the other hand, 5 strains of *Streptomyces* which produce broad-spectrum or antimitotic antibiotics displayed larger inhibition zones on the mutant strains than on the wild-type strain FL200, depending upon the antibiotics synthesized by

Table 6. Activity of collection strains producing known antibiotics in the screening system.

Strains	Antibiotics produced	Inhibition zone diameter (mm)		
		Strain FL200 (YPG agar)	Strain PR13 (YPG 80 agar)	Strain PRC24 (YPG 80 agar)
Streptomyces fungicidicus ATCC 27432	Nystatin	17	—	—
S. natalensis ATCC 27448	Pimaricin	22.5	10	7
S. rimosus ATCC 10970	Rimocidin and tetracycline	14	—	7
S. chattanoogensis ATCC 13358	Tennecitin	12	—	—
S. nodosus ATCC 14899	Amphotericins	11.5	—	—
S. griseus ATCC 11746	Candicidin	17	—	—
S. naraensis ATCC 13788	Cycloheximide	19	45	12
S. rimosus f. *paromomycinus* ATCC 14827	Paromomycin	10.5	23.5	20
S. hygroscopicus ATCC 27438	Hygromycins	9.5	16	15
S. lavendulae ATCC 8664	Streptothricins	13.5	25	21.5
S. coeruleorubidus ATCC 13740	Anthracyclines	7	31	31.5
S. verticillus ATCC 15003	Bleomycins	—	11.5	11

Antifungal activities of 7 day-old strains grown on solid media have been determined by the agar plug method (diameter of the agar cylinder: 5 mm).
—: No inhibition zone.

these collection strains.

We have screened for the production of antifungal activities by 3,000 strains of Actinomycetales from the laboratory collection which consists of *Streptomyces* (70%), *Micromonospora* (20%) and rare actinomycetes (10%). The antifungal activities of 7 day-old actinomycete strains, growing at 27°C on solid media, have been determined by the agar plug method, using *S. cerevisiae* FL200, PR13 and PRC24 as indicator strains. 311 strains (10.5%) have an inhibitory effect on *S. cerevisiae* FL200; among them 49 strains (1.6%) exhibit a polyene macrolide-like activity and 148 strains (4.9%) a cycloheximide-like activity. 114 strains (3.8%) are active on both the wild-type and mutant strains; a proportion of these actinomycete strains could co-produce polyene macrolides with another antibiotic exhibiting an antifungal activity. 103 other strains (3.4%) were found to be active only on the *S. cerevisiae* mutant strains PR13 and PRC24 and inactive on bacteria. An attempt to identify certain products isolated in the screen is in progress.

Discussion

Actinomycetes are well known for their ability to produce a wide variety of antifungal compounds, particularly polyene macrolides and cycloheximide. Different strategies can be employed to develop a profitable program to screen microbial metabolites for antifungal antibiotics while selecting against the polyene macrolide and cycloheximide producing strains. For instance, screening can be restricted to the detection of cell-wall acting antifungal compounds[11] detected by their ability to block the regeneration of *Neurospora crassa* protoplasts. Another approach is to search for new antifungal antibiotics which induce morphological changes in fungal and yeast cells[12], but this allows identification of polyene macrolides only after a butanol extraction of the active substances.

An alternative approach would be to use the mutant strain of *S. cerevisiae* PRC24, that is completely insensitive to the antifungal activities of polyene macrolides and cycloheximide in the presence of Tween 80; in contrast, this strain is highly susceptible to a large range of biologically active products, including antifungal, antitumor and antibacterial agents.

Polyene macrolide-resistance is mediated by a change in the sterol content of the cell membrane[7~9]; the high susceptibility of the isolated mutants to a large variety of antibiotics suggests a modification of cellular permeability resulting from a change in sterol composition. The fact that tetracycline, which has no activity against intact wild-type yeast cells but can inhibit protein synthesis by yeast ribosomes in a cell-free system[13], is active against the mutant strains supports this hypothesis. In the same way, yeast

strains with distorted cell membranes have been described to be inhibited by antibiotics which do not normally exhibit an antifungal activity only because they cannot enter the cell[14,15]. Our results suggest that the inhibition of the activity of yeast ribosomes in a cell-free system, as reported in the case of tetracyclines[13], can be extended to other families of antibiotics known to block protein synthesis, *e.g.* aminoglycosides. This increased cell permeability can also support the fact that antibiotics able to inhibit mitochondrial ribosomes, *e.g.* macrolides[16] are active on the mutant strains, although their antifungal activities are much less pronounced. Also, the change in sterol composition of the cell membrane seems to make it more fragile and indeed to make it more susceptible to antibiotics acting mainly on the cytoplasmic membrane, as polyethers, colistin or polymyxin B.

Strains of yeast which are more susceptible to cycloheximide and antitumor antibiotics have been described by GAUSE *et al.*[14], OKI[15] and SPEEDIE *et al.*[17]. Our mutants have been compared to *S. cerevisiae* strain FL599-1B, isolated by Dr. F. LACROUTE (Gif-sur-Yvette, France), which has been used for the screening of antitumor substances[14,15]. Strain NAP40b is identical to strain FL599-1B, with respect to its susceptibility to antimitotic antibiotics. In contrast, strains PR13 and PRC24 are much more susceptible to these compounds, and in addition are susceptible to various antibacterial antibiotics. The mutants we described are not selectively killed by antitumor agents, although they are supersensitive to some; therefore they may be useful in elaborating a screening method to discover such compounds but may also be used to discover other products. Particularly, strain PRC24 possesses two qualities essential in the search for non-polyenic antifungal agents: It is resistant to the polyene macrolides and cycloheximide, and it is highly susceptible to other antifungal antibiotics.

References

1) MARTIN, J. F. & L. E. MCDANIEL: Production of polyene macrolide antibiotics. Adv. Applied Microbiol. 21: 1~52, 1977
2) ZYGMUNT, W. A. & P. A. TAVORMINA: Steroid interference with antifungal activity of polyene antibiotics. Applied Microbiol. 14: 865~869, 1966
3) FUKUDA, H.; Y. KAWAKAMI & S. NAKAMURA: A method to screen anticholesterol substances produced by microbes and a new cholesterol oxidase produced by *Streptomyces violascens*. Chem. Pharm. Bull. 21: 2057~2060, 1973
4) GOTTLIEB, D.; H. E. CARTER, J. H. SLONEKER & A. AMMANN: Protection of fungi against polyene antibiotics by sterols. Science 128: 361, 1958
5) IANNITELLI, R. C. & M. IKAWA: Effect of fatty acids on action of polyene antibiotics. Antimicrob. Agents Chemother. 17: 861~864, 1980
6) HICKEY, R. J.: The antagonism between the antifungal antibiotic, ascosin, and some long-chain, unsaturated fatty acids. Arch. Biochem. Biophys. 46: 331~336, 1953
7) BARD, M.: Biochemical and genetic aspects of nystatin resistance in *Saccharomyces cerevisiae*. J. Bacteriol. 111: 649~657, 1972
8) WOODS, R. A.: Nystatin-resistant mutants of yeast: Alterations in sterol content. J. Bacteriol. 108: 69~73, 1971
9) MOLZAHN, S. W. & R. A. WOODS: Polyene resistance and the isolation of sterol mutants in *Saccharomyces cerevisiae*. J. Gen. Microbiol. 72: 339~348, 1972
10) KIDA, T.; S. TAKANO, T. ISHIKAWA & H. SHIBAI: A simple bioassay for herbicidal substances of microbial origin by determining *de novo* starch synthesis in leaf segments. Agric. Biol. Chem. 49: 1299~1303, 1985
11) KIRSCH, D. R. & M. H. LAI: A modified screen for the detection of cell wall-acting antifungal compounds. J. Antibiotics 39: 1620~1622, 1986
12) GUNJI, S.; K. ARIMA & T. BEPPU: Screening of antifungal antibiotics according to activities inducing morphological abnormalities. Agric. Biol. Chem. 47: 2061~2069, 1983
13) BATTANER, E. & D. VAZQUEZ: Inhibitors of protein synthesis by ribosomes of the 80-S type. Biochim. Biophys. Acta 254: 316~330, 1971
14) GAUSE, G. F.; A. V. LAIKO & T. I. SELESNEVA: Yeast mutants with distorted cell membranes as tests in the screening for antitumor antibiotics. Cancer Treatment Reports 60: 637~638, 1976
15) OKI, T.: New anthracyclin antibiotics. Jpn. J. Antibiotics 30 (Suppl.): S-70~S-84, 1977
16) GRIVELL, L. A.; P. NETTER, P. BORST & P. P. SLONIMSKI: Mitochondrial antibiotic resistance in yeast: Ribosomal mutants resistant to chloramphenicol, erythromycin and spiramycin. Biochim. Biophys. Acta 312: 358~367, 1973
17) SPEEDIE, M.K.; D. V. FIQUE & R. N. BLOMSTER: Radiometric prescreen for antitumor activity with a *Saccharomyces cerevisiae* mutant strain. Antimicrob. Agents Chemother. 18: 171~175, 1980