

Biological Characteristics of Antifungal Agents Produced by an *Actinomyces* Isolate

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Of a large number of *Actinomyces* spp. screened from soil for their antifungal activity, a strain referred to as G-10, identified as *Actinomyces naeslundii*, was found to produce potent antifungal agents against various species of fungi and even some bacteria. Even though it was originally isolated from natural environment, G-10 grew fairly well at 37°C as well as at 30°C. The maximum antifungal activity, however, was detected from 3~5 day shaking culture at 30°C. The initial pH value of 5.0 was the most optimal for both cell growth and antifungal activity. Different sources of carbon influenced only on cell growth and it is not likely that they have any direct correlation to antifungal activity. Soybean meal was a good nitrogen source for growth but had no direct stimulatory effect on production of antifungal agent. In general, nutritional requirement does not seem to have any direct effect for the activity. The principal inhibition was exerted via prohibiting the mycelial growth of filamentous fungi and budding in single cell fungi. The concentrated culture supernatant has anti-fungal activity equivalent to 20,000 U/ml nystatin. Pathogenic fungi were relatively more resistant than non-pathogenic ones. Self-inhibition, consequently the population growth inhibition, could be detected when they were densely grown on solid media.

KEY WORDS □ *Actinomyces naeslundii* G-10, antifungal activity

Before the era of antibiotics, the majority of antifungal agents were chemical substances, strong fungicides and surface antiseptics, inactivating vital enzymes by heavy metal ions with highly toxic effects on bacteria, protozoa, fungi, animals, and even plants (28). Some organic substances such as sulphur compounds, fatty acid, benzoic acid, phenol derivatives, quaternary compounds, and many others have shown more specific antifungal activities, but are limited to topical use only.

Discovery of antibacterial antibiotics and the increased prevalence of superinfections associated with such a fungus as *Candida* stimulated a vigorous search for antifungal antibiotics. Waksman and his colleagues, in 1952, recognized two groups of antifungal antibiotics (30); one group very active against fungi and bacteria was too toxic for the therapy, while the other also very active against fungi only. Discovery of nystatin, active in *Candida* infections, opened up a new era in the therapy of mycoses. After candididin was isolated and described, a number of reports on antifungal antibiotics had been accumulated (18).

All were very active *in vitro* against saprophytic or pathogenic fungi, yeast, or filamentous fungi

responsible for superficial or deep seated mycoses (26). Despite similarity in chemical structure, therapeutic effectiveness in experimental and clinical infections varies considerably, due to differences in such factors as solubility, diffusibility, toxicity, inactivation by serum components, and others (29).

Among these polyene antibiotics, only amphotericin B is absorbed, at least partially and without toxic effect, when administered orally (29). Another antifungal agent, hamycin, admitted orally, showed antifungal activity in patients with blastomycoses (7,27). When studied by various investigators, the mode of action of polyene was found to be an alteration of cell permeability, leading to leakage of potassium and metabolites essential for life (7,11), or to respiratory troubles blocking ATP regeneration (21).

Among the antibiotics produced by fungi, as first shown by Gentile (8) for human use, griseofulvin, first discovered in 1935 and applied only in plant fungal infections, is unique in being absorbed when administered orally; it is active against both superficial and systemic infections due to dermatophytes. The only antifungal antibiotic of bacterial origin, pyrrolnitrin, was

described (1), but its activity on pathogenic fungi is much weaker than that of amphotericin B, hamycin, or saramycetin. Now, amphotericin B is considered to be the sole compound of microbial origin available for systemic use (29). The various antibiotics so far isolated from culture of different microorganisms may be divided after Waksman into three broad groups on the basis of their respective antimicrobial spectrum (30), active either to fungi or to bacteria, or to both.

On the basis of some other discoveries on antifungal agents from soil microorganisms, an antifungal agent was detected from a strain of *Actinomyces* sp. We have studied on and further identified as *Actinomyces naeslundii*. We have confirmed its taxonomical niche and investigated some morphological and physiological properties, subsequently the antifungal activities.

MATERIALS AND METHODS

Strain

Soil samples were collected at Deogjeog Island, Kyunggi Province, Korea. These samples were diluted to 10^{-3} ~ 10^{-4} times with distilled water. Diluted samples were added to equal volume of phenol solution diluted to 1:140, then left to stand still for 10 minutes.

One loopful inoculum was transferred to starch casein medium (17,28), and the plates were incubated at 28~30°C for 5~7 days. Fifteen strains were isolated and their antibiotic activities were examined. Strain G-10 was selected since it showed the most potent antifungal activity and used throughout this experiment.

Identification of strain G-10

For identification of strain G-10, its morphological characteristics on various media and general physiological properties were examined. Subsequently, the taxonomical niche was determined according to classical taxonomic keys (15,17). The morphological properties were investigated on starch casein medium (17), glycerol arginine medium (4,20), yeast extract malt extract medium (22), and other laboratory media in general use.

In order to characterize the physiological properties of the strain, the conventional items in general use were tested. Especially the growth was observed using slide chamber culture method.

Mass culture

Mycelial inoculum of 1 ml/ suspension was transferred to 100 ml/ proper culture medium in 500 ml/ Erlenmeyer flasks, and incubated at 30°C for 7~10 days on a rotary shaker at 200 rpm. For the mass culture of strain G-10, a variety of synthetic medium was tested. Nutrient broth initially adjusted to pH 5.0 was proved very

optimal for cell growth and production of antifungal agents, hence selected for further experiments. With the appropriate time intervals, supernatant was tested for antifungal activity using target organisms listed in Table 1. Most of the microorganisms used in this experiment were originally provided by Korean National Institute of Health and the others were from the stock culture of Dr. E.C. Choi, College of Pharmacology, Seoul National University. They were kept in our laboratory thereafter.

Effects of nutrient sources for carbon and nitrogen were evaluated with different concentration of various sugars and soybean media (50~200 mg/ml), respectively, in Gause mineral salts medium I (4,14).

Assay of antibiotic susceptibility of strain G-10

The antibiotic susceptibility of G-10 itself was tested to evaluate sensitivity to several antibiotics commercially available such as penicillin, streptomycin, clotrimazol, and nystatin by a paper disc agar diffusion method (2). The amount loaded on each disc was 2,000 unit, 2 mg, 2 mg, and 200 unit, respectively.

RESULTS AND DISCUSSION

Description of morphological and physiological characteristics

Strain G-10 used in this experiment was identified as *Actinomyces naeslundii* on the basis of the morphological and physiological characteristics, with a broad spectrum of antimicrobial activity (Table 1). Strain G-10 was Gram-positive, non acid-fast, non-sporulating, and non-motile. Its microcolonies in slide chamber culture on brain heart infusion agar (BHIA) have a dense mass of filaments, at their centers surrounded by a periphery of radiating, curved and branched filaments of 1~3 μ m in width which gave the diphtheroid cells, the typical shape of *Actinomyces naeslundii* filament. The 7-day culture of macrocolonies of strain G-10 on BHIA were 3~5 mm in diameter, round with undulated margin. They were frequently heaped or irregularly lobate and might resemble the molar-tooth colony of *A. israelii* as shown in Fig. 1.

Aerial mycelium was not frequently observed. As no spore was developed, induction of sporulation has been tried thereafter with various methods. The onset of differentiation in actinomycetes is very often associated with the establishment of limiting conditions for vegetative growth. Presumed switching mechanism for secondary metabolism is indeed very similar to those for sporogenesis (16,23).

Although industrial strains of *Actinomyces* certainly belong to categories of regulatory mutants, some minor quantities of antibiotics are usually produced by cultures freshly isolated from

Table 1. Microorganisms used for evaluation of antibiotic activity for G-10

Target microorganisms	Inhibition zone
<i>Allescheria boydii</i>	12 mm
<i>Aspergillus nidulans</i> A6	8.5
<i>Aspergillus niger</i> 337	20
<i>Candida albicans</i> YU1200	17
<i>Fusarium</i> sp.	8
<i>Geotrichum</i> sp.	16
<i>Hansenula</i> sp. R-1	12
<i>Hansenula</i> sp. Y-2	14
<i>Hansenula</i> sp. Y-3	24
<i>Hansenula</i> sp. Y-4	17
<i>Microsporium gypseum</i>	12
<i>Mucor</i> sp.	8
<i>Neurospora</i> sp.	12
<i>Penicillium</i> sp.	17
<i>Scopulariopsis</i> sp.	6
<i>Trichophyton ajelloi</i>	5
<i>Trichophyton mentagrophytes</i>	3
<i>Trichophyton tonsurans</i>	9.5
<i>Trichophyton verrucosum</i>	11.5
<i>Bacillus subtilis</i> IAM 1069	7
<i>Escherichia coli</i> ATCC 11105	4
<i>Klebsiella pneumoniae</i> ATCC 10031	—
<i>Lactobacillus leichmannii</i> ATCC 7080	—
<i>Micrococcus flavus</i> NCIB 8166	—
<i>Mycobacterium smegmatis</i>	—
<i>Pseudomonas albicans</i> ATCC 10490	—
<i>Serratia marcescens</i>	—
<i>Staphylococcus aureus</i> ATCC 6538p	—
<i>Staphylococcus epidermidis</i> ATCC 12228	—

Antimicrobial activity has been evaluated by paper disc diffusion method. The growth inhibition zone was determined as a distance in mm from edge of the paper disc to the point of microbial growth. Total volume of 100 μ l culture supernatant of G-10 in nutrient broth for 4 days was loaded onto the paper disc (5 mm in diameter) where target organisms were previously spread. Plates were incubated at 37°C.

the natural environment (9). It is, indeed, in practice to have actinomycete cultures back to soil when they lose ability to differentiate as the result of laboratory manipulations. It is, however, evident that there are positive correlations between the ability to form aerial mycelium and spores and some of the secondary metabolites. Strain G-10 also in a certain aspect showed a kind of positive correlation between pigmentation, characteristic earthy smell, and antifungal activity, especially on BHIA and yeast extract malt extract (YM) medium as in some *Streptomyces* spp. meanwhile few has been reported on antifungal agents from *Actinomyces* spp. Morphological characteristics of strain G-10 on various media

and physiological ones for taxonomy are described in Tables 2 and 3, respectively.

Mass culture and antifungal activity of strain G-10

Typical features of mass culture is summarized in Fig. 2. G-10 was cultured in nutrient broth, and cell mass, pH changes, and antifungal activities were checked every day for a week and 10th day of culture. The antifungal activity has been tested against *A. niger* 337. The antifungal activity, however, showed slight difference in different cultures. As shown in Fig. 2, changes in cell mass, pH, and antifungal activity were dramatically increased for the first 2~3 days, while those changes were not so great afterwards. Initial pH was examined for the desirable increase in cellular mass maintaining the proper antifungal activity, finding that the pH of 5.0 ± 0.1 , before autoclaving, gave the maximum cell mass with relatively high antifungal activity. Cells were grown throughout the experiment on the basis of this finding. Each culture with different pH converged to pH 7.5~8.2 after 10 days of culture regardless of their initial pH.

Temperature should be one of the most important factors influencing cellular growth (6, 25). As to the cell growth, 37°C was the best while the highest antifungal activity was observed at 30°C with proper humidity. Antifungal activity during the culture has been checked every day against some target organisms at 30°C on Pen-assay medium (0243 Difco, antibiotic assay broth), and the results were expressed in the diameter of inhibitory zone produced by strain G-10. Total cell mass from 100 ml culture for 7 days using different media had been measured in dry weight, and antifungal activities of each culture was estimated against different kinds of microorganisms (Tables 4 and 5). Cell growth does not have any correlation to antifungal activity.

Even though the quantitative use of inhibitory zones of agar was used as early as 1885 (30) to evaluate the inhibition of the growth of one microorganism by diffused substances produced by another organisms, diffusion disc tests with antifungal agents are not fully standardized yet (3, 5, 6, 12, 13, 24, 29) and the application is rather limited in such cases as polyene compounds for their instability (13) and imidazoles for insolubility (10). Sometimes, ceramic cylinder was preferred to paper disc diffusion method, but it had been confirmed that these two methods were not significantly different (3, 5, 10, 19, 29). Considering the sequence of events which may occur when an antibiotic diffused through the agar matrix in which microbial cells capable of multiplying in the medium are present, it will be evident that consideration must be given to diffusion, growth rate, adsorption, permeability partition and specific antibiotic action. The concentration of initial cells and the vegetative

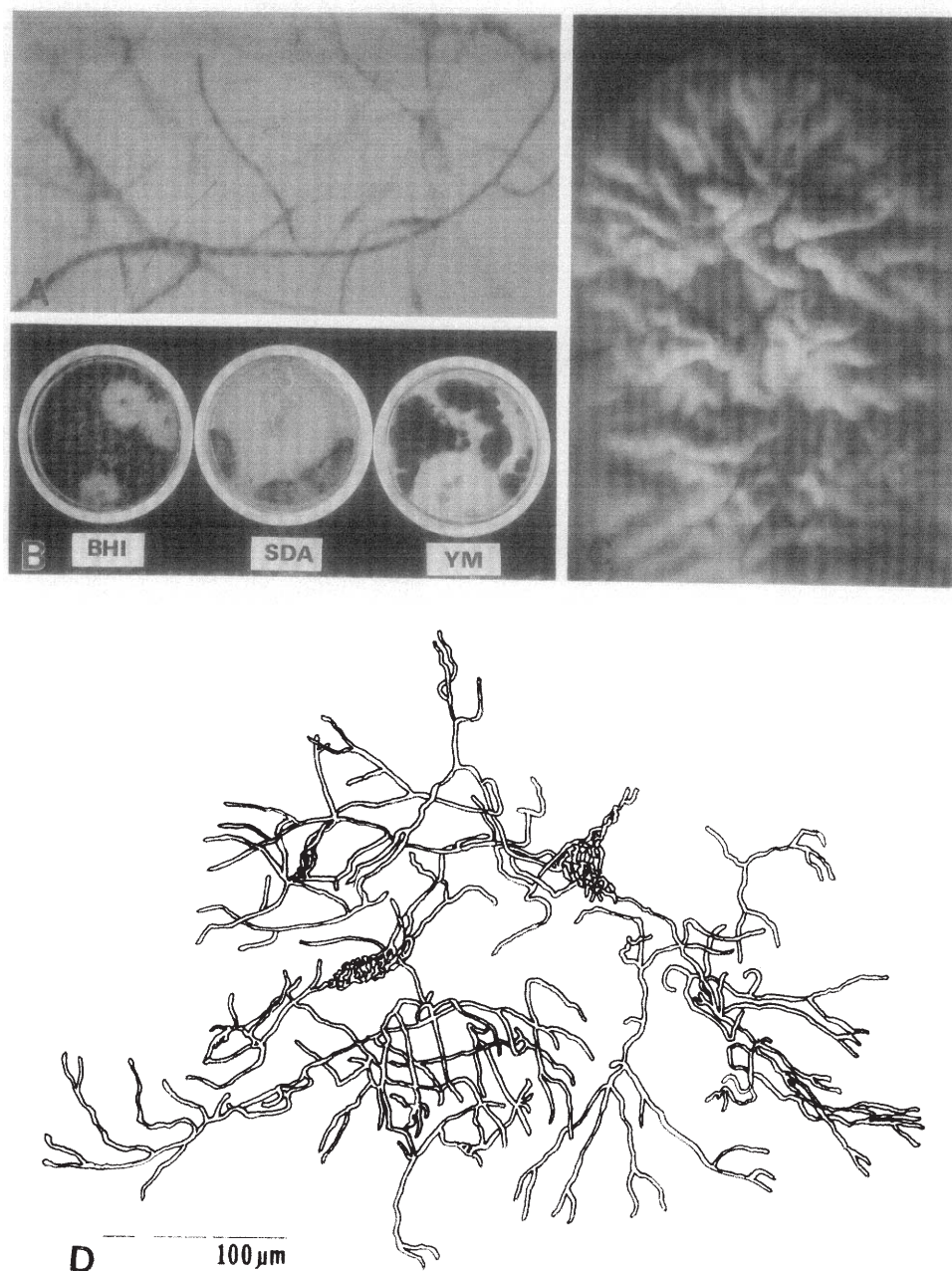


Fig. 1. *Morphological characteristics of strain G-10.*

- A. Microscopic observation reveals curved and branched filaments of diphtheroid forms after Gram staining.
- B. Strain G-10 clearly has typical molar-tooth type colonies on conventional agar media. BHI, brain heart infusion agar; SDA, Sabouraud dextrose agar; YM, yeast extract-matose extract agar.
- C. Close observation of the typical molar-tooth colony of strain G-10.
- D. Strain G-10 has been cultured in a slide culture chamber on BHI agar for detailed structure of filamentous growth.

Table 2. Morphological characteristics of strain G-10 on various media.

Media	Cultural characteristics		
	colony	aerial mycelium	pigment
Brain heart infusion agar (BHI)	round, entire, deep brown	+	dark brown
Nutrient agar (NA)	round, undulate, faint yellow	—	
Yeast extract-malt extract agar (YMA)	round, undulate, yellow brown	—	dark brown
Sabouraud dextrose agar (SDA)	round, entire, hollow, yellow	—	dark brown
SFM2 (Gause mineral salt medium I)	round, lacerate, yellow brown	+	
SFM3 (Pridham and Gottlieb trace salt solution)	round, undulate, light brown	+	
Kligler iron agar	walnut, undulate, grayish black	+	
Czapek-Dox agar	round, undulate, lacerate	+	
G-arginine agar	round, undulate, yellow brown	—	
Peptone yeast extract glucose agar	highly lacerate	—	
Soluble starch agar	white	+	
Starch casein agar	creamy	+	
Synthetic agar	round, white	+	

stages of fungal growth made it more complicated to develop a standard assay method. These complex factors must be considered again with completely purified antifungal agent. At present, the assay recognized and in current use by British Society for Mycopathology is regarded as standard (2).

Nutrient requirement of strain G-10

Strain G-10 utilized various kinds of sugars and carbohydrates for carbon sources which also influenced the production of antifungal agent. Carbon sources on the base of utilization and effects in mass culture are listed in Tables 6 and 7, respectively. These carbon sources were, therefore, evaluated the effect on antifungal activity using some test organisms. Soybean meal

Table 3. Major physiological characteristics of strain G-10.

Motility	—
Growth at 4°C	—
20°C	w
30°C	+
37°C	++
40°C	+
50°C	—
Spore	—
H ₂ S formation	—
Catalase	—
Oxidase	—
Staining, gram	+
Acid-fast	—
Spore	—
Gelatin liquefaction	—
Agar liquefaction	—
Starch hydrolysis	—
Nitrate reduction	—
Indole formation	—
MR-VP test	—

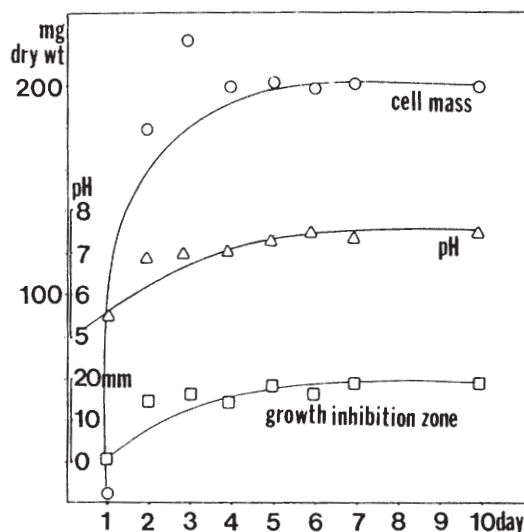


Fig. 2. Typical features of mass culture of strain G-10. Cells were grown in 100 ml-shaking culture in SD broth medium at 30°C, 200 rpm. Changes in cellular mass, pH, and antifungal activity against *Aspergillus niger* 337 were examined.

was also tested as a nitrogen source for the antifungal activity. Soybean meal was a good nitrogen source for the cell growth but not so good for the production of antifungal agent. **Concentration and antimicrobial spectrum of the antifungal agent**

Table 4. Growth of strain G-10 on various media and antimicrobial activity.

Media	Dry weight (mg/100 ml, shaking culture)	Antifungal activity ^a					
		A	C	Ta	Tm	S	B
PYG broth	691.9	7	5	17	3	2	2
BHI broth	472.1	4	2	5	3	4	2
Starch casein broth	317.5	5	1	3	2	4	2
SD broth	198.4	9	8	7	1	3	2
Nutrient broth	189.4	8	2	3	3	6	7
Synthetic broth	170.7	8	5	2	4	7	12
Glutamate assay broth	115.6	2	5	7	5	2	6
G-arginine broth	96.9	12	3	7	4	3	2
Soluble starch broth	73.5	4	3	5	2	2	1

^a Antimicrobial activity has been evaluated by paper disc diffusion method using target organisms. A, *Aspergillus niger* 337; C, *Candida albicans* YU1200; Ta, *Trichophyton ajelloi*; Tm, *Trichophyton mentagrophytes*; S, *Scopulariopsis* sp.; B, *Bacillus subtilis* IAM1069. The growth inhibition zone was determined as explained in Table 1.

Table 5. Antimicrobial activities evaluated during mass culture.

Target microorganisms	Day					
	2	3	4	5	6	
<i>Candida albicans</i> YU1200	7	7	6	7	5	
<i>Trichophyton ajelloi</i>	10	13	18	15	17	
<i>Trichophyton mentagrophytes</i>	10	12	17	17	15	
<i>Scopulariopsis</i> sp.	5	7	12	12	8	
<i>Aspergillus niger</i> 337	21	20	22	20	20	
<i>Bacillus subtilis</i> IAM1069	9	8	10	8	8	

Culture supernatant from 100 ml-shaking culture of strain G-10 at 30°C in nutrient broth (initial pH 5.0) was collected everyday until day 6 and evaluated its antimicrobial activity against target microorganisms listed. The supernatant was concentrated using freeze-drying and antimicrobial activity was expressed by the same way as explained in Table 1.

The culture concentrate of extracted antifungal agent by the method of Seo and Hong (24) showed a high activity corresponding to 20,000 U/ml activity of nystatin. Hence the broad spectrum against various kinds of fungi and even some bacteria such as *Bacillus subtilis* IAM 1069 and *Escherichia coli* ATCC 11105 (Table 8). To confirm the antibacterial and antifungal activity, some commercial antibiotics were compared with those of strain G-10. Since the amount loaded on the paper disc was different for each drug, the activities cannot be compared directly, but it is very likely that pathogenic fungi are more sensitive to the antifungal agent of the strain G-

Table 6. List of sugars utilizable or unutilizable for strain G-10 as sole carbon sources.

Utilizable: starch, sorbitol, inulin, glucose, inositol, salicin, maltose, sucrose, cellobiose, dextrin
Unutilizable: mannitol, ribose, adonitol, galactose, xylose, saccharose, raffinose, citrate, dulcitol

Each sugar has been evaluated in Gause mineral salt medium I.

Table 7. Effect of carbon sources for antimicrobial activity.

Carbon source	Antimicrobial activity					
	S	A	C	Ta	Tm	B
D-Galactose	1	2	5	7	3	1
Sorbitol	5	4	4	3	7	2
Inositol	7	7	2	8	2	2
D-Mannose	5	4	4	8	4	2
Maltose	4	2	2	18	2	— ^a
L-Arabinose	3	5	5	3	7	2

Various carbon sources were examined for their effect on antimicrobial activities. Strain G-10 was cultured in Gause mineral salt medium I supplemented with 0.5% of each carbon source at 30°C for 5 days, and the culture supernatant was tested by paper-disc diffusion method. Antimicrobial activity was evaluated using the same target organisms used in Table 4.

^a Not detected.

10 than non pathogenic ones. Each drug was prepared from salt-form.

Many pathogenic fungi attack human, animals, and even plants. With few exceptions, most of the fungi pathogenic to man are considered as fungi imperfecti. The modes of their attack might be classified into superficial, cutaneous and systematic mycoses. Many reviews report different aspects of action mechanisms (27). As a biological agent, product of the strain G-10 might have a broad activity against a large number of microorganisms. Cycloheximide, nystatin, pimarin, filmarisin, amphotericin B, hamycin, levorin, and others are the antifungal antibiotics with a broad spectrum against a variety of yeast-like and filamentous fungi (11).

Extraction of the antifungal agent

Further extraction was tried with chloroform and ethyl ether obtaining very fine needle like creamy crystals which showed growth inhibition against *A. niger* even though it was a very weak response, suggesting that further purification and concentration are required. The same sample was

Table 8. Comparison of the antimicrobial activities of strain G-10 with commercially available antifungal drugs.

Target micro-organisms	Clotri-mazol 1 mg	Griseo-fulvin 4 mg	Nys-tatin 500 unit	Nifu-ratel 1.25 mg	Strain G-10 4d-culture in BHIB NB	
<i>Allescheria boydii</i>	10	7	1	15	4	7
<i>Aspergillus niger</i> 337	5	6	4	5	10	12
<i>Trichophyton mentagrophytes</i>	7	3	2	6	15	4
<i>Geotrichum</i> sp.	8	3	3	5	4	5
<i>Aspergillus nidulans</i> A6	4	4	1	6	12	5
<i>Aspergillus usami</i>	2	1	2	3	4	3
<i>Bacillus subtilis</i> IAM1069	13	1	1	1	6	7

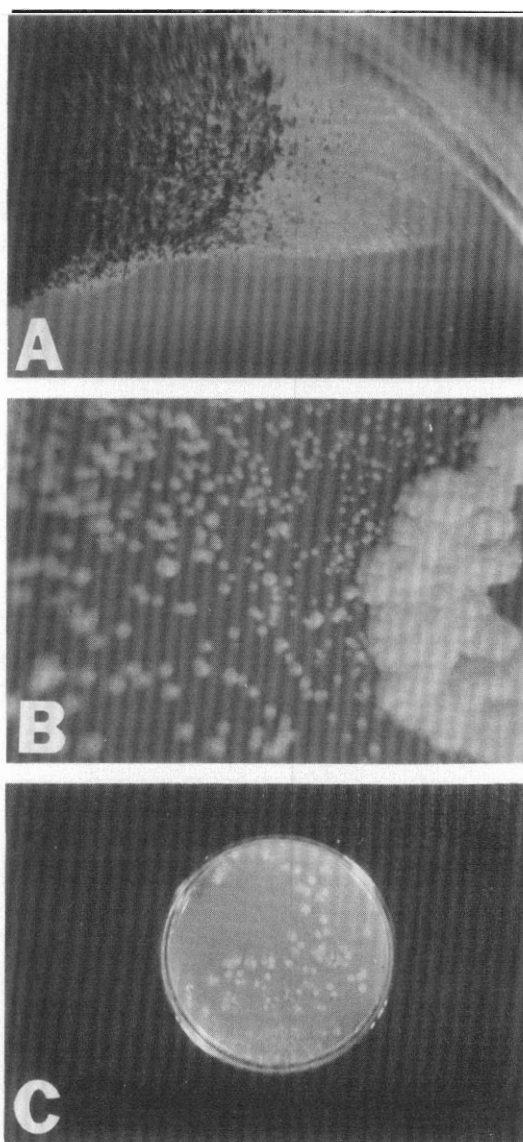
Commercially available antibiotics with antifungal activity have been compared with those of strain G-10 grown in brain heart infusion broth (BHIB) and nutrient broth (NB) for 4 days and each supernatant was further concentrated as explained in the text. Doses of commercial antibiotics were diluted as shown so that proper inhibitory zone could be compared with culture supernatant of G-10.

purified by TLC method. Culture supernatant was concentrated and eluted in methanol-butanol-water (40:20:40) system. The fractions were assayed for the antifungal activity and Rf value determined was 0.1. Evaluation of antifungal activity of each fraction was, however, carried out with the limited amount due to the small capacity of paper discs, and the activities should be re-examined after final purification.

Mode of action

The filamentous fungi were greatly inhibited (Fig. 3A), while the yeast-like fungi were relatively resistant (data not shown). The fungal filaments showed no growth on solid media where the antifungal agent from G-10 was already diffused even though they differentiated into spore forming stage from the mycelia grown before diffusion of the antifungal agent. In the case of yeast-like fungi, however, the cells on the region where the antifungal agent was already present showed no budding at all.

Strain G-10 also showed a kind of self-inhibition on most of the media, which is typical in *Actinomyces* spp. Early investigators noticed that accumulation of certain antibiotics above the critical level would exhibit or completely suppress vegetative growth of the producing organisms (30).

**Fig. 3.** Characteristic antibiotic activities of strain G-10.

A. Growth of filaments of *Aspergillus niger* 337 is inhibited by antifungal agent produced by strain G-10. The differentiation into sporulation is not blocked.

B. Self-inhibition of own growth was observed when strain G-10 was reinoculated near the colonies grown 3 days ahead.

C. Population-inhibitory mode also could be noticed since the colonies are much smaller where the population is denser.

Extensive use of this finding was applied in selection for strains with elaborated activities (Fig. 3).

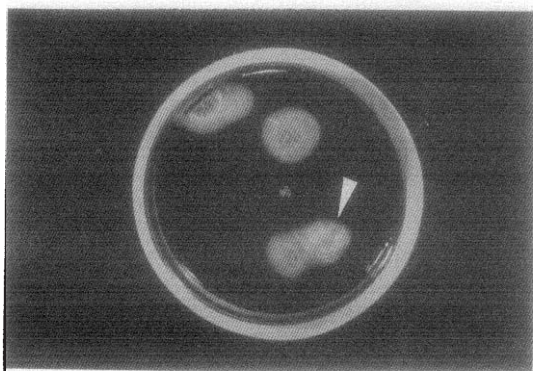


Fig. 4. Amylase activity.

Strain G-10 (arrowhead) does not secrete amylase and antifungal activity was not affected by the enzyme secreted by *Aspergillus niger* 337.

Table 9. Antibiotic susceptibility of strain G-10.

Antibiotics	Inhibitory zone
Penicillin (2,000 units)	19 mm
Streptomycin (2 mg)	16
Clotrimazol (2 mg)	23
Nystatin (200 units)	— ^a

^a Not detected.

In conclusion, this agent could be considered to inhibit the cell wall synthesis as the yeast-like fungi are more resistant than the filamentous ones suggesting the differences of the cell wall composition. It might be too toxic for the therapeutic use because as *Actinomyces naeslundii* was reported to be isolated from oral cavity (15) but with smooth type colonies supposed to be nonpathogenic. So this powerful agent should be purified furthermore and its critical antifungal activity should be modified for the effective use.

Amylase production by strain G-10

Amylase production has been tested whether this is correlated with antifungal activity, according to the conventional method on starch medium (Gause mineral salts medium I supplemented with 2% glucose) to see if strain G-10 secretes amylase. For control *Aspergillus niger* 337 was grown in the same plate. It is not likely that strain G-10 produces amylase. The amylase from *Aspergillus niger* 337 does not deteriorate the antifungal agent as shown in Fig. 4.

Antibiotic susceptibility of strain G-10

Inasmuch as the antibiotic susceptibility of *Actinomyces naeslundii* has not been reported, the strain G-10 was assayed for antibiotic sensitivity to some antibiotics such as penicillin, streptomycin, clotrimazol, and nystatin. As expected,

nystatin could not inhibit the growth of strain G-10. The result of the susceptibility test was summarized in Table 9.

Sporulation

Sporulation was induced by UV irradiation and nutritional starvation. UV irradiation was carried out with different doses varying irradiation time from 1 to 30 min at a fixed distance of 10 cm from the lamp (G15T8, Sylvania, GTE, 15W), and starvation effect was tested on poor nutrition media (SFM2 and SFM3) (23, 25). Sporulation, however, was not easily induced with UV irradiation nor with nutritional starvation (data not shown). Sporulation might require some factors other than those we have tried.

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초 록: *Actinomyces* 분리군주에 의하여 생성된 항진균제의 생물학적 특성

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토양에서 분리한 수 종의 *Actinomyces* spp. 가운데에서 진균의 생장억제물질을 분리하는 군주를 분리하고, 그 성장 및 생리특성을 조사하여 이를 *Actinomyces naeslundii* G-10으로 동정하였다. 실험에 사용한 군주 G-10은 섭씨 37도에서 가장 양호한 성장을 보였으나, 진균생장억제 능력은 30도에서 더 강하였다. 배지의 초기 pH는 5.0으로 조절하여 주었을 때 성장과 진균 생장억제물질의 분비가 양호하였으며, 진균생장억제능력은 진탕배양에서 3~5일만에 최고치에 달하였다. 진균생장의 억제에는 paper-disc agar diffusion 방법으로 조사하였다. 배양이 끝난 뒤 배양상등액을 농축하고 부분적으로 정제한 시료는 nystatin 20,000 단위/ml에 해당하는 역가를 보였다. 그러나 정확한 역가의 비교는 완전한 정제가 선행되어야 가능하다고 판단된다. 탄소원과 질소원으로 공급된 물질들은 진균 생장억제물질의 분비에 직접적인 영향을 미치지 못하였다. 균사를 가지는 진균들은 균사의 성장이 억제되었으며, 효모형의 진균들은 증식하지 못하였다. G-10의 군집이 있는 주위에 다시 G-10을 접종하면 이들의 증식이 억제되고 있음을 관찰하였으나, 이들의 생장저지가 진균생장억제물질에 의한 것인지는 확인되지 아니하였다.