THE ANTIBIOTIC AND ANTITUMOR ACTIVITIES OF SELECTED PHILIPPINE THALLOPHYTES

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Abstract

Out of a total of 905 Streptomycetes studied, 466 showed varying degrees of activity against the gram positive, gram negative and acid fast bacteria, the yeast and yeast-like organisms, plant pathogens and Ehrlich ascites tumor cell.

Streptomyces culture S-62-30, S-67-3 were isolated from soils. The active brew was produced in a large scale by submerged fermentation. The antibiotic substance was isolated and purified and was identified as belonging to the Actinomycin group. *In vitro* studies showed significant activity against ascitic tumors, *Mycobacterium* 607 and the gram positive bacteria. *In vivo* studies in Swiss mice showed inhibition of Ehrlich ascites carcinoma (EAT) and leukemia L1210. Isolate S-A-15, a natural variant of S-62-30, was identified as *S. parvus*, Philippine strain. It also produced an actinomycin complex with same activity as the parent strain.

An initial survey of 172 basidiomycetes gave 169 sporophore aqueous extracts which inhibited mainly the gram positive test bacteria. Subsequent samples also indicated the same inhibitory activity.

Five basidiomycete species namely *Polyporus cinnabarinus, P. sanguineus, Lentinus squarrusulus, Calvatia lilacina* and *Psathyra umbonata* were successfully grown in the laboratory and were found to produce the antibiotic substance.

Some 40 basidiomycete samples were shown to have inhibiting property on Ehrlich ascites tumor cell (EAT).

Ether extracts of Sargassum samples and *Chlorella pyrenoidosa*, showed inhibitory activity on the gram positive bacteria. Some species gave indication of antitumor activity.

Out of 33 lichens, 30 showed antibiotic activities against gram positive and *Mycobacterium* 607. Usnic acid, an antitubercular substance, was isolated in pure form from lichens *Usnea montagnei, Ramalina farinacea* and *Crocynia membranacea.* Polysaccharides from the first two of these lichens showed promising results when tested on Leukemia L1210 and P 388 experimental tumors.

Introduction

Going over the discovery of antibiotics, one can observe that practically every discoverer was a man ripe in years. Almost all discoveries in the antibiotic field have been made by man between the ages of 45 and 60. This fact must please us all — the YOUNG, because it confirms that scientific creation has no age limit; the MATURE, because it proves that they can still accomplish. The maturity of antibiotic scientists is a symbolic herald of our geriatric era.

Dr. Benjamin Duggar, 76 years old, led a team of Lederle scientists and isolated the organism *Streptomyces aureofaciens* which yields the golden antibiotic aureomycin. The late Dr. Selman A. Waksman, soil microbiologist and professor of Microbiology in Rutgers University, at about the age of 65, screened more than 10,000 isolates of *Actinomyces* and *Streptomyces* before he discovered *Streptomyces griseus.* The microorganism is now commercially used for the production of an antibiotic known as streptomycin.

Antibiotic research has changed. Originally there were innumerable lone investigators but today the work is done in teams. Also the fact that a discovery is a lucky accident has been replaced by discovery as the result of planning and organization. It has been fifty two years since the discovery of penicillin by Fleming and thirty two years since the broad spectrum antibiotics became available for clinical use. Let us note the difference between the two achievements of the antibiotic age. The first was accomplished by one man alone as in the days of Pasteur; the second by a team of men dedicated to tracking down an antibiotic. This marks the development of a new mental and social attitude in research.

Brief History of Antibiotics and Definition (7, 18)

A brief history of antibiotics is traced back to more than a century ago. In 1876, Tyndall, a physicist, described the antagonistic action of a species of *Penicillium* to bacterial growth. A year after in 1877, the first scientific demonstration of bacterial antagonism was recorded by Pasteur and Joubert. In 1889. Vuillemin used the term "antibiosis" to describe antagonism among bacteria, for the first time. Olitsky reported the antagonism of *B. fluorescens liquiefaciens* towards *Staphylococcus* strains and *B. anthracis* in 1891. Muljutin (1893) described inhibition of growth of a number of microorganisms by *Vibrio cholerae.* Metchnikoff (1894) and Kitasato (1889) noted the strong antagonism of *Pseudomonas aeruginosa* toward *Vibrio cholera.* In 1889 Emmerich and Low isolated from *Pseudomonas aeruginosa,* pyocyanase the first drug produced by microorganisms and introduced into therapy. A seldom mentioned scientist Ernst Augustin Duchesne preceded Fleming by about 31 years. In 1897 he presented his doctoral thesis on the antibacterial action of Hypomycetes. He demonstrated by experiment that cultures of *Penicillium glaucum* decreased the virulence of *Bacillus coli* or *Eberthella typhosa* in inoculated animals. In 1908, the antimicrobial properties of molds were recognized by rural folks. They treated a small cut or wound with a piece of mold, resembling a ball the size

of a lemon found in fields and pastures. Usually the grandmother kept these molds in bread or rye ready to apply to infected wounds and sores of the children. It was recorded that in 1911 -1913 a laboratory attendant collected molds growing in old pieces of shoe leather, made this into a salve and used this as medicine for wounds. In 1928 Sir Alexander Fleming noticed a species of penicillium that made Staphylococcus colonies surrounding it transparent. In 1939 Rene Dubos undertook methodical, biological and chemical studies on the antibiotic Tyrothricin produced by *Bacillus brevis*.

1939-1959 marks the golden era of antibiotic research. The turning point in the history of antibiotics was the isolation of penicillin by Florey *et al.* (1939) from cultures of *Penicillium chrysogenum*. The period was called the Golden Era of antibiotic research. It was during this time that the main therapeutic antibiotics presently used namely benzyl penicillin, streptomycin, chloramphenicol, tetracycline and erythromycin were discovered.

The antibiotic era and the period preceding it are an outstanding phase in the history of medicine during which impressive advances in theoretical and applied science were made.

1959-1960 — Chemical modifications of natural antibiotic molecules were studied to obtain new, semisynthetic and modified antibiotics.

After 1959 interest in chemotherapeutic antibiotics waned and renewed in 1965.

Definition of Antibiotics

The first definition of antibiotics was that of Waksman (1945), who defined antibiotics as "chemical substances produced by microorganisms possessing the ability to kill or to inhibit the growth of bacteria and other microorganisms".

In 1960 Abraham and Newton proposed this definition, "Antibiotics are compounds of natural origin, produced mainly by microorganisms, characterized by high activity against pathogenic microorganisms, relatively low toxicity for humans and animals, and resistant to inactivation by enzymes and body fluids".

However, in 1976, Abraham and Newton's definition was modified (Kurlowicz *et al.)* as follows. "Antibiotics are natural compounds produced mainly by microorganisms, or are compounds obtained by chemical or microbiological modification of natural compounds".

Origin of Antibiotics

In the search for new antibiotics, it became apparent that, although microbes are the main producers of these compounds,

other organisms such as algae, lichens, green plants and even animal cells produce antibiotics.

More than 50% of all antibiotics (7) are elaborated by actinomycetes (Streptomycetaceae) 10 to 11% by fungi imperfecti (Aspergillales) and nearly the same number by bacilli (Bacillaceae-Bacillus). About 6% of antibiotics are synthesized by Basidiomycete and ascomycetes, less than 1% by lichens, and about 14% by green plants. About 2% of antibiotics are of animal origin. (Figure 1).

The search for antibiotic substances is world-wide and microorganisms producing them have been discovered in several countries. Table 1 gives the national trends in antibiotic discovery. Figure 2 shows that the highest number of antibiotic producing strains belong to the Streptomycetaceae (7).

Production of Antibiotics

About 2,700 antibiotic substances were produced by microorganisms, approximately 85% of those for therapeutics were produced by actinomycetes, 11% by fungi and 4-5% by bacteria. In 1978 the world-wide bulk sales of the 4 most important groups of antibiotics — the penicillins, the cephalosporens, the tetracyclines and erythromycin amounted to $4.2 billion.

A list of important antibiotics produced by fermentation processes for the year 1977 is given in Table 2.

Local Importation of Antibiotics

Since the time antibiotics were discovered and marketed our importation of antibiotics has progressed to enormous amounts. Central Bank figures show that from 1970 up to 1974 the amount was steady up to about 6 M dollar mark while in the succeeding 5 years there was a progressive annual increase of 1.5 to 3 M dollar until importation reached almost the 20 M dollar mark in 1979. Figure 3 shows the trend. This value comprises about 25% of the total Philippines imports of both medicinal and pharmaceutical products. Drug surveys show that majority of sales fall in the category of antibiotics and antibiotic preparations.

Local Attempts at Establishment of an Antibiotics Fermentation Plant

Way back in 1951 the establishment of an antibiotic fermentation plant in the Philippines was the subject of a bill passed by Congress. Unfortunately it did not pass the Senate so this bill did not materialize.

In 1969, the administration officials showed an interest in the establishment of a plant and Mr. Lopez, the V.P. at that time

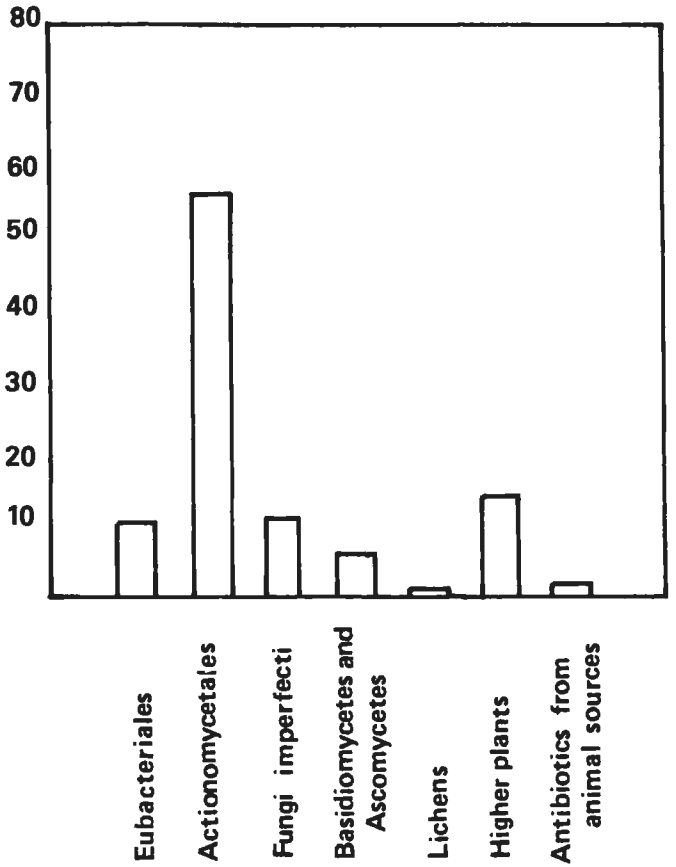


FIGURE 1 ORIGIN OF ANTIBIOTICS

exhorted traders on this particular investment area.

The Saturday Mirror Nov. 8, 1969 quoted:

"V.P. exhorts traders in investment areas"

. . . Antibiotics. Lopez indicated deep interest in the establishment of antibiotic fermentation plants to supply the big domestic demand. He pointed out that the total supply of the all-important drug in the local markets is wholly imported. Lopez said that with antibiotic fermentation plants here, the country will save no less than $26 million annually. The vice president also said that the establishment of these drug plants will complement plans for an expanded national health program. "I have complete faith in the capacity of our drug firms here both foreign and Filipino-owned to go into this important venture," he said.

Table 1. Showing National Trends in Antibiotic Discovery.

Country	Producing microorganisms				Total
	Streptomycetes sp.	*Rare actinos*	*Fungi*	*Eubacteria*	
Japan	1210	98	308	236	1852
USA	677	162	244	163	1246
USSR	261	42	34	39	376
UK	48	8	206	46	308
West Germany	110	6	28	23	167
Switzerland	68	1	84	1	154
Italy	53	47	14	6	120
France	58	2	32	27	119
India	55	13	18	30	116

It was about the same year that the United Laboratories planned to put up an antibiotic fermentation plant for some antibiotics. In addition, Rachelle Laboratories had a full page publication in one of the local dailies about the antibiotic fermentation plant that they planned to build in the Philippines.

The sad fact is that to-date more than thirteen years have passed, and our dreams of such a plant is not yet realized. It is hoped that at some future time some progressive drug companies will enter into this venture.

Problems Facing the Antibiotic Era

The new problems facing us in this antibiotic era are 1) antibiotic resistance 2) metabolic attack 3) biological symbiosis, 4) new sources of antibiotics. Another present-day problem is to find antibiotics that will attack the microbic forms of life still immune to them. The answer might be to discover new kinds of metabolic attack against such organisms, to study more about their biological cycles, or to develop a greater biological immunity in the human body. We might also end up by reducing to the essentials the drugs now being used, which reminds us of Osler's words: "The young physician starts life with 20 drugs for each disease, and the old physician ends life with one drug for 20 diseases". This reminds me of the late Dr. Daniel de la Paz, a U.P. Pharmacology professor in the 1930's, whose students commented that he prescribed only one medicine (i.e. sodium citrate) for all diseases.

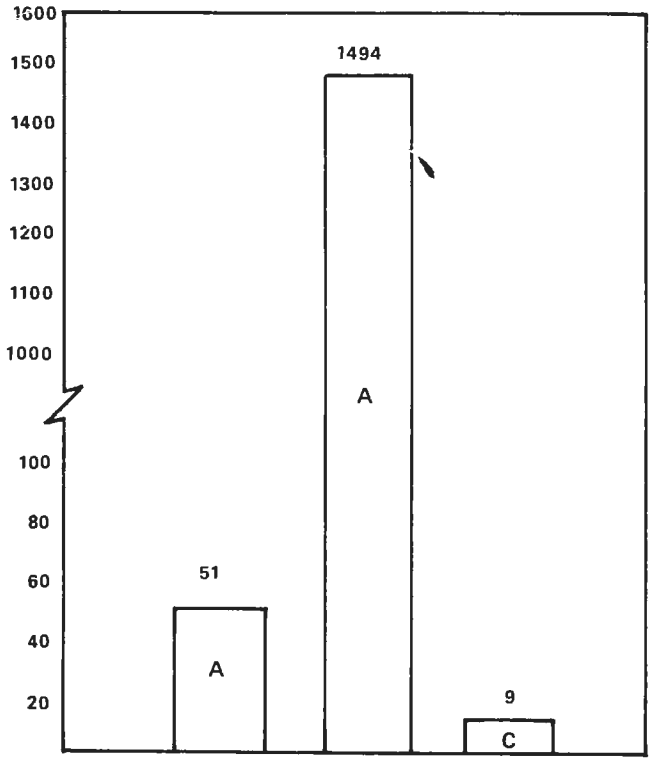


Figure 2. Showing the number of antibiotic-producing strains in the Actinomycetaceae, Streptomycetaceae and Actinoplanaceae

Legend:

A — Actinomycetaceae
- Actinomyces — 21
- Nocardia — 30

B — Streptomycetaceae
- Streptomyces 1467
- Thermoactinomyces — 6
- Micromonospora — 21

C — Actinoplanaceae
- Actinoplanes — 3
- Streptosporangium — 6

In the search for new sources of antibiotics, it may be mentioned that the human body and its intercellular or inner sea and humors probably contain defensive powers. It is also necessary to verify how and where the antibiotics work and how much of their action is exerted on the microbe and how much on the surrounding cells and humors. One may ask, "Do antibiotics create a system of chain reactions involving the antibiotic, the microbe and the humoral system?

Table 2. List of Antibiotics Produced by Fermentation (1977)

Adriamycin	Griseofulvin	Pristinamycins
Amphomycin	Hygromycin B	Quebemycin
Amphotericin B	Josamycin	Ribostamycins
Avoparcin	Kanamycins	Rifamycins
Azalomycin F	Kasugamycin	Sagamicins
Bacitracin	Kitasatamycin	Salinomycin
Bambermycins	Lasalocid	Siccanin
Bycyclomycin	Lincomycin	Siomycin
Blasticidin S	Lividomycin	Sisomicin
Bleomycin	Macarbomycin	Spectinomycin
Cactinomycin	Mepartricin	Streptomycins
Candicidin B	Midecamycin	Tetracyclins
Candidin	Mikamycins	Chlortetracyline
Capreomycin	Mithramycin	Demeclocycline
Cephalosporins	Mitomycin C	Oxytetracycline
Chromomycin A	Mocimycin	Tetracycline
Colistin	Monensin	Tetranactin
Cycloheximide	Myxin	Thiopeptin
Cycloserine	Neomycins	Thiostreptin
Dactinomycin	Novobiocin	Tobramycin
Destomycin	Nystatin	Trichomycin
Enduracidin	Oleandomycin	Tylosin
Erythromycin	Oligomycin	Tyrothricin
Fortimicins	Paromomycins	Uromycin
Fumagillin	Penicillin G	Validamycin
Fungimycin	Penicillin V	Vancomycin
Fusidic acid	Penicillins (semisynthetic)	Variotin
Gentamicins	Pentamycin	Viomycin
Gramicidin A	Pimaricin	Virginiamycin
Gramicidin J (s)	Polymixins	

Now-a-days there is a growing interest in the utilization of Philippine Medicinal Plants as shown by numerous researches that are now being heavily funded and conducted on the higher plants. What have we done about our lower plants as source of medicinals? Fresh sources of antibiotics should be developed. The cure for many infections may perhaps lie in the depths of the sea or in the unexplored Thallophytes.

Thus, we enter into the subject of my talk, "The Antibiotic and Antitumor Activities of Selected Philippine Thallophytes".

This work summarizes over 20 years of investigations on the biological activities of bacteria, fungi, algae and lichens. The selected species namely the streptomycetes, basidiomycetes, algae and lichens were tested for their inhibitory activities. Some species were worked out to isolate and characterize the chemical components responsible for their antibiotic activities.

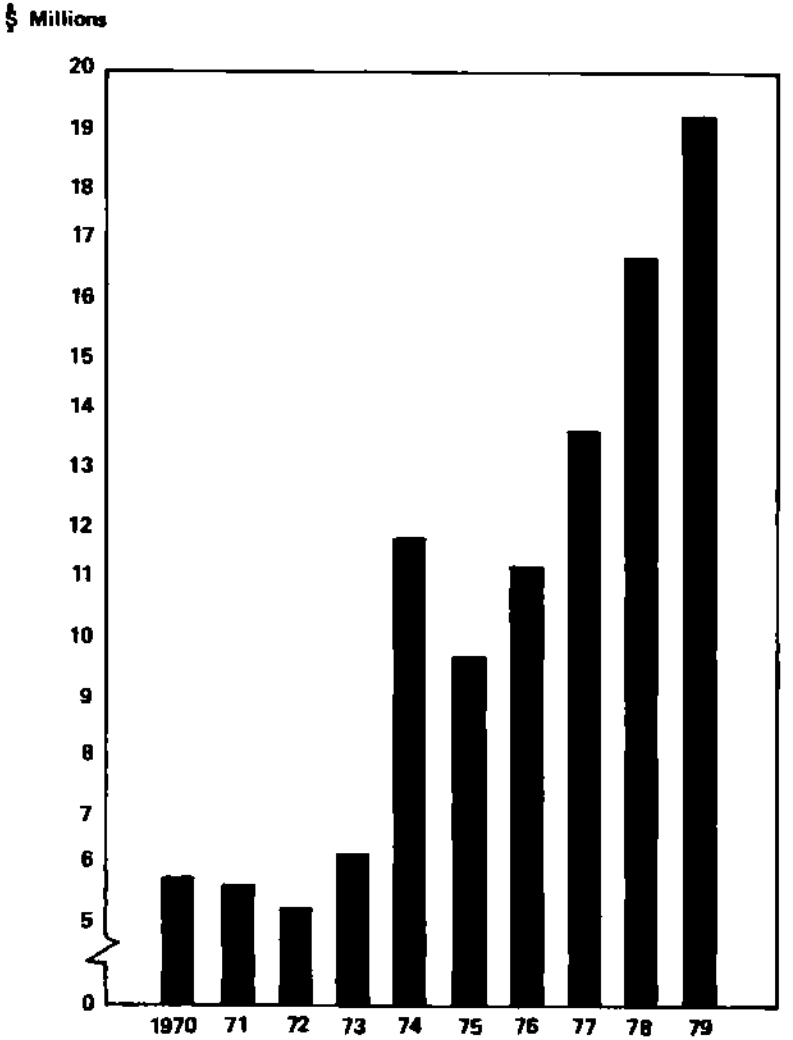


Figure 3. Graph Showing the Importation of Antibiotics from 1970-1979

Figure 3a. Graph showing the importation of individual antibiotics from 1970-1979

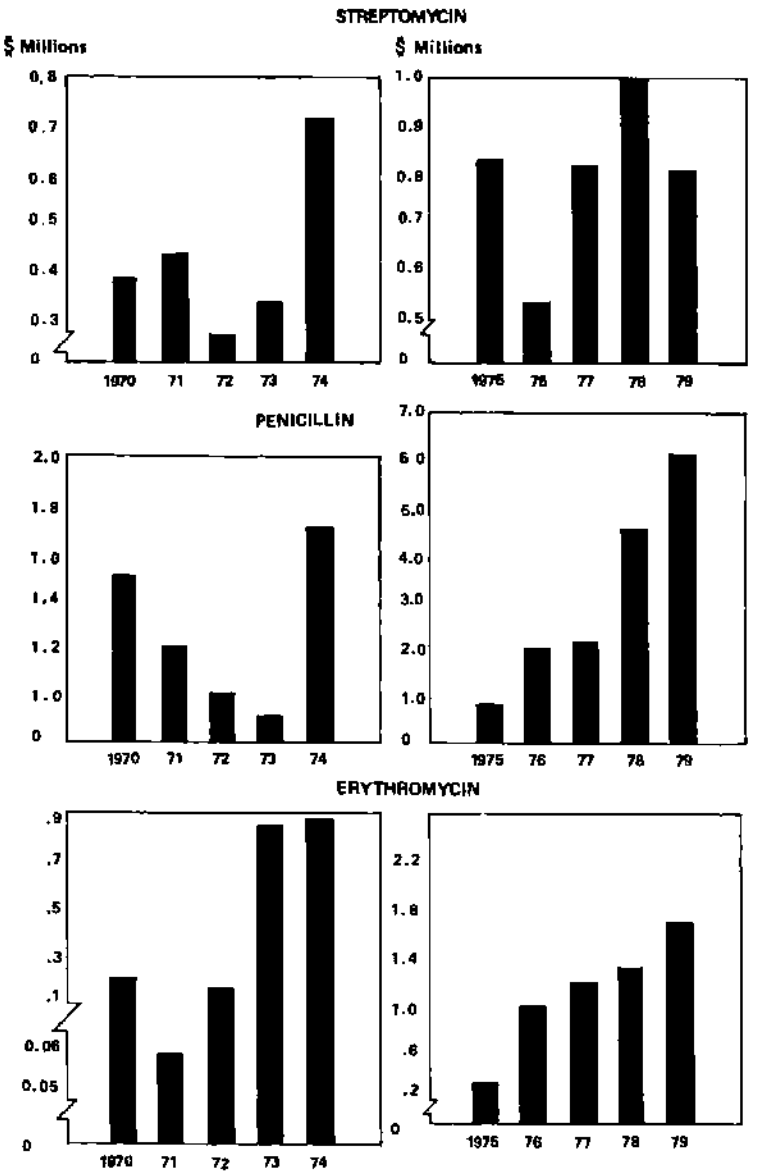
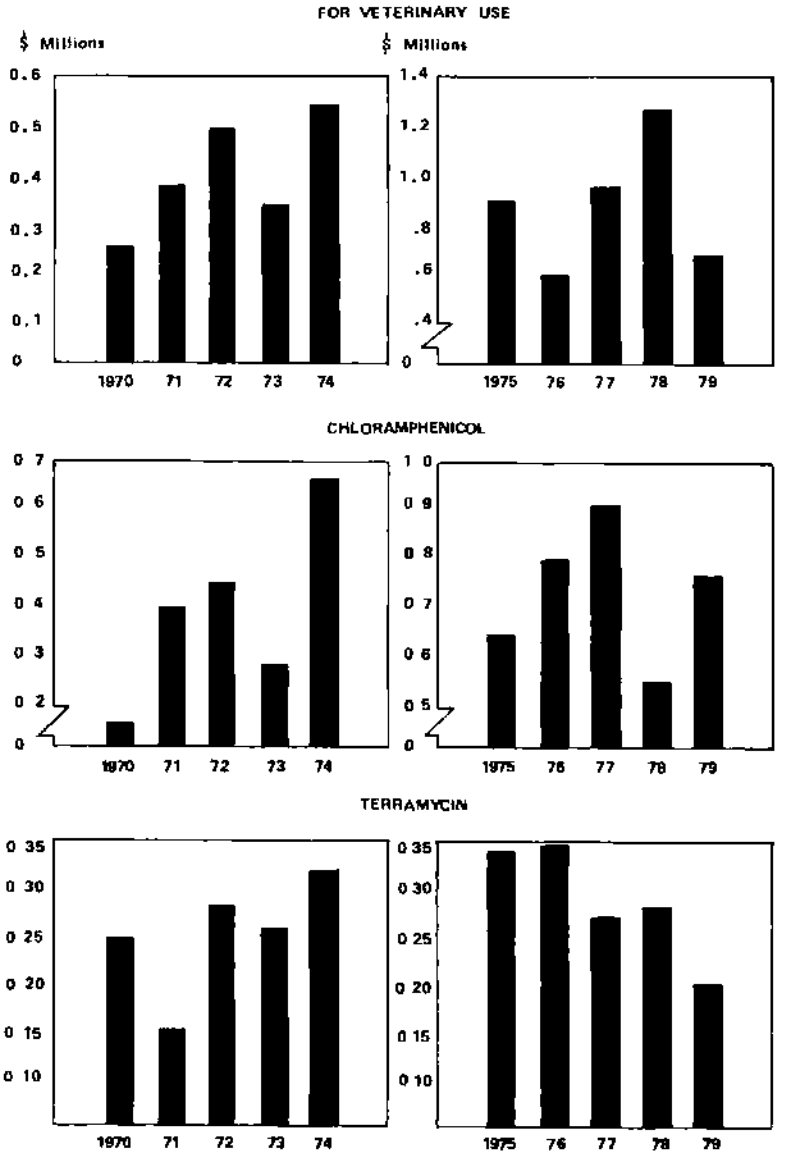


Figure 3b. Graph showing the importation of individual antibiotics from 1970-1979



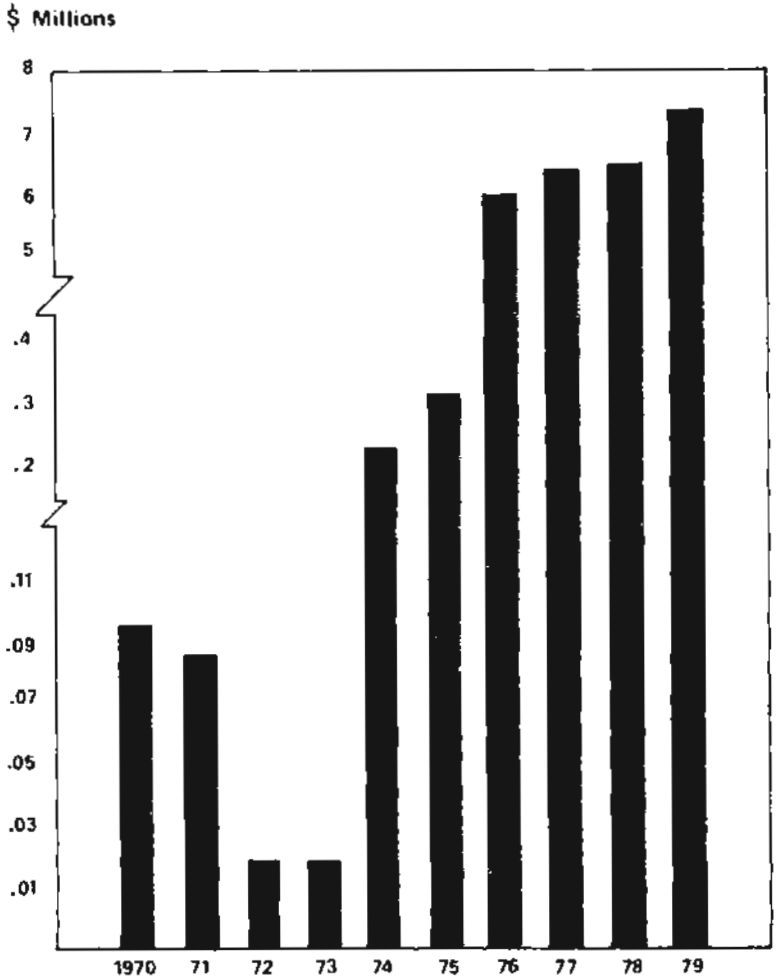


Figure 3c. Graph showing the importation of other antibiotics.

Methods of Testing and Test Organisms

Any of the following methods were used for the screening tests: On solid media (a) cross streak (b) agar plug (11).

On liquid media: Diffusion methods using a) stainless steel cylinder b) paper disc c) agar well d) broth dilution.

These assay methods have been reported previously. For the isolation of the streptomycetes from soil samples, any of the following methods were used: 1) simple dilution and plating out technique (11) 2) agar piece method (14) 3) selective method by antibiotic incorporation (15).

The following test organisms and tumor systems were used:

Gram positive: *Micrococcus pyogenes* var. aureus *209* P, *M. var. aureus* (Penicillin resistant), *Bacillus subtilis* FDA 219,

Gram negative: *Escherichia coli*, *Salmonella gallinarum* (Copenhagen strain), *Pseudomonas aeruginosa*, *Alkaligenes faecalis*, *Paracolon aerogenic.*

Acid-fast: *Mycobacterium* 607.

Yeast and fungi: *Candida albicans*, *Saccharomyces cerevisiae*, *Ceratostomella paradoxa*, *Fusarium moniliforme*,

Plant pathogenic fungi: *Pyricularia oryzae and Xanthomonas oryzae.*

Experimental tumors: Ehrlich ascites tumor cell, Leukemia L 1210 (L1210), P 388, Sarcoma S-180 (S-180).

I. Streptomycetes

For this study members of the genus Streptomycetes have been selected as the representative of the Bacteria. Attention to this group of organisms has increased over the past several years because almost all antibiotics except a few are produced by them.

Streptomycetes are a group of branching unicellular organisms belonging to the family Streptomycetaceae now generally accepted as bacteria. They were once considered as fungi by the mycologists and as higher bacteria by the bacteriologists or even as an intermediary group between the fungi and the bacteria. They are found in large numbers in the soil, in fresh waters, in lake and river bottoms, in dust, in manure, in compost and even on food products. They are generally aerobic, some are anaerobic and other spores are microaerophilic.

The search for potential antibiotic producers among microorganisms is worldwide and has been active for more than 50 years. The antibiotic properties of actinomycetes have been thoroughly investigated by several researchers, among them are Waksman (17), Benedict (2), Schatz and Hazen (16), Chua (4), Landerkin (8), Celino (3) and Sevilla-Santos (11-15).

It is noteworthy to mention that *Streptomyces erythreus (9, 20) and S. filipinensis* (22) which were both isolated from Philippine soil samples are producers of the antibiotics Ilotycin or Erythromycin, and filipin, respectively.

The initial survey of Philippine Streptomycetes was carried out in surface cultures in agar media and secondary screening in submerged cultures in shake flasks and production of large amounts in stirred and aerated fermentors.

An initial screening of 294 streptomyces cultures (11) in solid medium gave 80 potential antibiotic producers with anti-

biotic spectrum of activity against *M. pyogenes aureus* 209 P. and *B. subtilis* FDA 219. The sensitivity of *Ps. aeruginosa* to the action of the antagonistic actinomycetes was quite remarkable. For although reported to be resistant to known antibiotics, it was found to be more sensitive than *E. coli* and *S. gallinarum*. Forty six (57.5%) was found inhibitory to *Paracolon aerogenic*, a hemolytic strain of paracolon isolated at the Bureau of Research and Laboratories. Fifty six (70%) and 41 or (51%) of the active isolates were found inhibitory to *S. cerevisiae* and *C. albicans* respectively. Thirty three (41%) were inhibitory to *Myco* 607. The most active isolates and their sources are given in Table 3.

Ninety (90) cultures of streptomyces were grown in submerged cultures in shake flasks and 60 active cultures were obtained (12). The total number of cultures that are active against at least one test organism is given below:

Test organisms	*No. of cultures*
Gram positive bacteria (at least one)	51
Gram positive (3 of them)	17
Gram positive and gram negative	16
Yeast and filamentous fungi	23
Mycobacterium 607	28
Ehrlich ascites tumor cell	26

In these tests only 1 kind of tumor cell was used. It is advisable to test the active brew not only on one kind of tumor but on several experimental tumors such as sarcoma 180, carcinoma 755 and leukemia L-1210.

Only one, S-62-15, had strong activity against gram positive and gram negative bacteria besides having antitubercular activity.

The production of antibiotics against plant pathogens (14) *Xanthomonas oryzae* and *Pyricularia oryzae* was surveyed in solid (primary screening) and also in submerged culture (secondary screening). It was found that of 272 streptomyces in solid medium, 160 showed inhibitory action of varying degrees against either of the two plant pathogens or both; 36 against *X. oryzae* only and 39 against *P. oryzae*, and 85 against both pathogens. Submerged culture of 52 isolates using 8 media combinations gave 29 strong antibiotic producers, 47 moderate and 11 weak ones. It was observed in the media studies that there seemed to be a medium really suitable for antibiotic production for any particular isolate.

Selective isolation of streptomycetes (15) by incorporation of antibiotics into the screening media and fermentation in submerged culture gave a total of 249 streptomycetes from 171 soil samples. Chlortetracycline, oxytetracycline and tetracycline 2

Table 3. Showing the Most Active Streptomyces Isolates, Their Sources and Activity

Isolate No.	*Origin of Soil*	*Degree of inhibition of active streptomycetes against test bacteria*							
		I	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*
5779	Batangas 2	++++	+++	+++	++	++	+++	++++	++++
5731	Batangas 3	++++	++++	—	—	—	+++	+	+
5751	Compost, SLH	+++	++++	—	++	+	—	++++	—
5579	Albang, Rizal	+++	+++	+++	++++	+++	++	+	+
5787	Batangas 4	++++	+++	—	—	—	+	++	+
5724	Batangas 4	++++	+++	++	++	—	—	—	—
5719	Quezon City	+++	+++	++	++	—	+++	++	++
5780	Sulu	+++	+++	—	—	—	+++	++	++
5618 A	Papaya, N.E.	+++	+++	+	—	—	+	+++	++
5614	Peñaranda, N.E.	+++	++++	—	—	—	+++	—	—
563	Diliman, Q.C.	++++	—	—	—	—	—	—	—
57108	Balete, Aklan	+++	+	++	—	—	—	—	—
5746	Balete, Aklan	+++	+	—	—	+	—	—	—

Legend:

I — *Micrococcus pyogenes aureus* 209P
II — *Bacillus subtilis*
III — *Escherichia coli*
IV — *Salmonella gallinarum*
V — *Pseudomonas aeruginosa*
VI — *Mycobacterium* 607
VII — *Saccharomyces cerevisiae*
VIII — *Candida albicans.*

Inhibition zones

++++ — 30 mm and above
+++ — 15 — 29 mm
++ — 5 — 14 mm
+ — 5 mm and below

mg/ml (0.5 ml) per plate with 0.5 ml of statin were used as treatment for all plates. In solid medium, 64 produced vitamin B_{12}; in liquid medium, 143 produced 1 to 120 μg vitamin B_{12} per 100 ml. fermentation brew. The antibiotically active isolate in solid and liquid media were 166 and 94 respectively. Of the former, 14 were active against all test microoganisms and of the latter only two were active.

Table 4 gives the summary of the screening of the streptomycetes included in this report.

In this survey of the antibiotic activities of streptomyces from Philippine soil samples, several promising organisms were obtained and a few, namely, S-62-30, S-67-3, S-A-15, S-27-C were studied in detail (6a, 13, 15a). Actinomycins were isolated from the culture of the first three of these and oxytetracyline produced by the last one. Chemical analysis of the complex S-62-30 did not show any threonine which was present in S-67-3. *In vitro* studies showed significant activity against ascitic tumors *Mycobacterium* 607 and gram positive bacteria (11). *In vivo* studies in Swiss mice

Table 4. Showing Number of Antagonistic Streptomycetes

No. of Soil Source	*Culture Method*	*No. of Streptomycetes Tested*	*Total Active Culture*	*Number of cultures active against individual groups of test organisms*					
				Bacteria			*Yeast & Fungi*	*Plant Pathogens*	*Tumor System*
				I	*II*	*III*	*IV*	*V*	*VI*
30	solid	294	80	55	24	33	56		
182	shaken	90	60	34	17	29	20		26
—	solid	272	160					39	
—	liquid	52						19	
171	solid	249	166	119	53	NT	NT		
171	liquid			68	9	NT	NT		

Legend:

I — *M. pyogenes var. aureus*
II — *E. coli*
III — *Mycobacterium 607*
IV — *S. cerevisiae*
V — *P. oryzae*
VI — *Ehrlich ascites tumor cells (EAT)*

NT — *not tested*

showed inhibition of Ehrlich ascites carcinoma (EAT) but not sarcoma (S-180) nor Leukemia (L-1210) using Actinomycin D as standard (1).

S-A-15 was identified as *S. parvus* Philippine strain (13). When cultivated in a dextrose-peptone-meat extract medium by shake flasks and stirred aerated jars, actinomycin complex was obtained. The isolation procedure is given in Figure 4.

Part III. Basidiomycetes (Higher Fungi)

One of the most promising research projects which is believed to have a vital relation and significance to the problems of drug research, drug industry especially so in a country like ours which imports drugs, is an investigation into the possibilities of Philippine basidiomycetes (mushrooms and toadstools) as potential sources of medicinals, drugs and antibiotics.

The Basidiomycetes or commonly called higher fungi are those fungi in which the sexual spores are borne externally on special club-shaped cells called *basidia*, the edible ones being called mushrooms and the poisonous ones referred to as toadstools.

They are plants belonging to the lowest of all plant groups, the Thallophytes. Each grows tiny reproductive body called a spore which is formed in the mature plant. In some mushrooms, millions of spores are formed in gills beneath the cap; some in

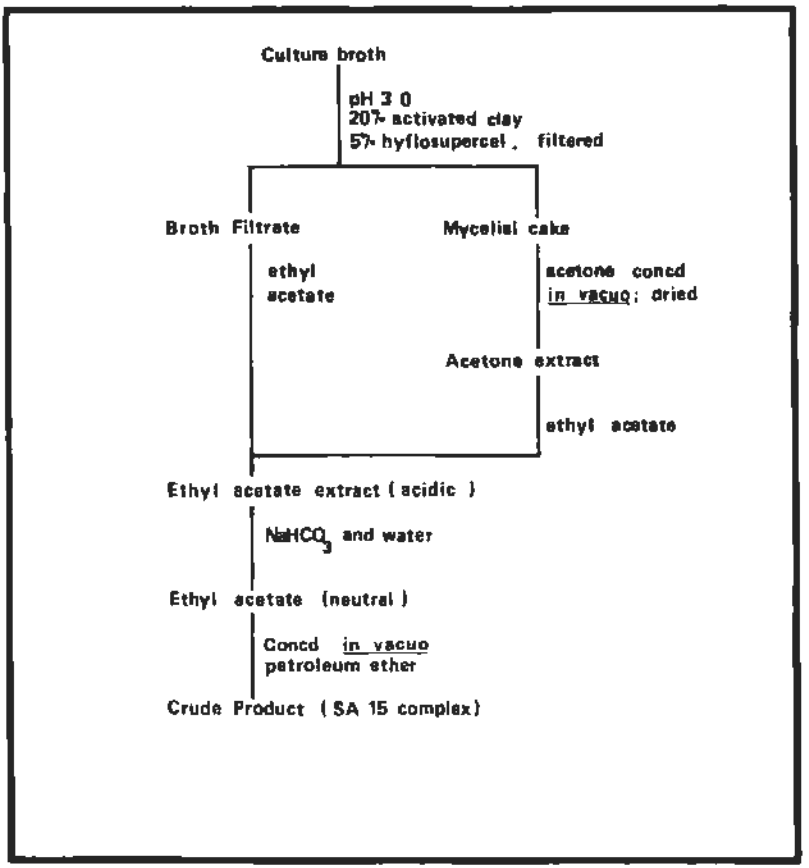


Figure 4. Schematic diagram of method of isolation of an antibiotic produced by streptomycete (SA—15 complex)

pores or tubes or in corrugated walls under the cap; some in the mass of white flesh comprising the whole fungus.

As food for man, mushrooms have an ancient history. However, mushrooms were not only used for food. Their medicinal uses either real or fancied, continued to exist for a long time. The old Greeks had used pieces of burning Fomes or Polyporus to cauterize wounds and ulcers. The latter became known as the magical Cure-all, a marvelous mushroom believed to have more power over the ills of mankind than anything else at that time. Its power covered various ailments such as broken bones, sores, colic, bruise, liver complaints, asthma, jaundice, dysentery, kidney trouble, hysteria, epilepsy, and snake bites. By the beginning of the 17th century the old beliefs continued. Dried puffballs were used to stop bleeding and fumes from a burning puffball were employed for their slightly anaesthetic property during operations. The Jew's ear mushroom was used for throat inflammations.

Even as late as 1884, *Lactarius* was eaten as a fancied cure of tuberculosis in England. The fly Amanita besides being effective in killing flies, was indicated for epilepsy, ringworm infection and used as a narcotic. In the Philippines the mature puffballs locally called "Tumbong" are reported to be used to stop bleeding. Table 5 gives a list of some mushrooms and the diseases for which they are indicated.

Table 5. Some Medicinal Mushrooms, and Uses (18)

Name of mushroom	*Indications*
Fomes (burning)	cauterize wounds and ulcers
Polyporus (burning)	cauterize wounds and ulcers
also for:	broken bones, sores, colic, bruise, liver complaint, asthma, jaundice, dysentery
Puffball (dried)	stops bleeding
Puffball fumes	anaesthetic during operations
Jew's ear	throat inflammations
Lactarius	tuberculosis (England)
Fly amanita	killing flies, epilepsy, ringworm narcotic
Puffballs	stop bleeding (Philippines)

The importance of basidiomycetes as source of antibiotics came into focus as early as 1944 when Wilkins and Harris (24) made an examination of Basidiomycetes for the presence of bacteriostatic substances. Among the studies made along this time in several parts of the world were those of Mathieson (9), Robbins *et al.* (13), Hervey (17) and Atkinson (2), Sevilla-Santos *et al.* (17, 20).

Out of the numerous antibiotic substances produced by the mushrooms and toadstools, only a few have been studied extensively and were proven valuable. Lofren *et al.* produced nebularin from *Agaricus nebularis* and found it active against human, bovine and avian mycobacteria. Psalliotin was extracted from *Psalliota xanthoderma* (1). Clytocybin was first described by Hollande. It was obtained from *Clitocybe gigantea* var. *candida* and found to inhibit *B. typhosus*, E. coli, *Brucella abortus* and *Myco. tuberculosis*. Pleurotin was extracted from *Pleurotus griseus* and was reported to inhibit *S. aureus*, *B. mycoides*, *B. subtilis*, *E. coli*, *Myco. phlei*, *Myco. smegmatis*, *Myco. tuberculosis* and *K. pneumoniae*.

From *Polystictus sanguineus* collected in India Bose (3) isolated an antibiotic polyporin which inhibits *S. aureus*, *Str.*

pyogenes, *Str. viridans*, *S. typhi*, *S. paratyphi A*, *S. paratyphi B*, *Bact. coli*, *V. chlórea* and *Sh. flexneri* (4). The culture fluid was filtered aseptically, placed in ampoules and used successfully in the treatment of typhoid fever. Antibacterial substances have likewise been reported in other higher fungi of India. Meyer (11, 12) reported that extracts of *P. cinnabarinus* (Jacq.) Fries inhibited *Staphylococci*, *Streptococci* and *Pasteurella avicida*.

Other antibiotic compounds from mushrooms are given in Table 6.

Table 6. Antibiotic Compounds from Mushrooms

Antibiotic compounds	*Basidiomycete source*
Pleurotin C_{20} H_{22} O_5	*Pleurotus griseus*
Biformin	*Polyporus biformis*
Biforminic acid	*Polyporus biformis*
5-methoxy-p-toluquinone	*Coprinus similis and Lentinus degener*
Marasmic acid	*Marasmius conigenus*
Nemotin	*Poriacorticola*
Nemotinic acid	*Poria tenuis*
Agrocybin	*Agrocybe dura*
Polyacetylenes	*Clithcybe distrata*
Illudin R	*Clitocybe illudens*
Illudin S	
Pleuromutilin C	*Pleurotus mutilus*
C_{22} H_{34} O_5	*Pleurotus passeckerianus*
Antibacterial substances	*Drosophila substrata*
Fomecin A	*Fomes juniperinus*
Polyporin	*Polystictus sanguineus*
Cinnabarin	*Polyporus cinnabarinus*

Oncostatic principles were produced *in vivo* and *in vitro* by species of the genus Calvatia which later was called calvacin (8, 14). From *P. botulinus*, triterpenes were isolated and were found effective on malignant neoplasms. (22, 23).

An initial survey of 172 Basidiomycetes tested showed 169 with varying degrees of activity on the test organisms (17). It was found that the gram positive bacteria were more sensitive to the action of the mushroom extracts than were the gram negative test bacteria.

The next survey included 587 Basidiomycetes samples (20) which were tested for their activity against 3 gram positive and 4 gram negative bacteria (20). Out of 587 samples, 506 were found to inhibit at least one of the test organisms used. 60.48 per cent were observed to contain substances inhibitory to *M. pyogenes* and *aureus* 209 P; 59.11 per cent to penicillin resistant

M.P. aurens: 18.74 per cent to *S. gallinarum*; 27.43 per cent to *P. aeruginosa*; and 40.2 per cent to *A. faecalis*. The figures indicate that the gram positive bacteria are more sensitive to the inhibitory action of the basidiomycete extracts than to the four gram negative ones. A list of the oustanding species is given in Table 7.

It was observed that the inhibitory action of extracts from various samples occurs not only in different species but also in the same species collected from same places at different periods of the year.

Since it is definitely known that Basidiomycetes are potentially a good source of antibiotics, the problem that may arise in a possible commercial exploitation of the valuable substances is *where* and *how* to get a big and continuous supply of the fungus raw material. The natural supply is out of the question as it can not be expected to be consistently large and continuous. Cultivating the Basidiomycete under natural conditions or in green houses may entail much expense without any certainty that it would always produce sporophores containing the inhibitory substances. If a strongly active antimicrobial substance from Basidiomycetes could only be produced in the same way as penicillin is produced from *Penicillium chrysogenum* or streptomycin from *Streptomyces griseus*, then the problem of its commercial production would not be difficult to solve.

It was observed that a few species were found to produce the antibiotic substance both in the sporophore in nature, and also in the culture liquid of the fungus grown in the laboratory. Two wood rotting fungi *P. sanguineus* and *P. cinnabarinus* Phil. sp. which were cultivated in mineral salts solution with rice bran (darak, Tag.) extract produced very potent antibiotic with antibacterial and antitumor activities. A yellow substance very similar to that obtained by Bose in India was isolated from them by extraction and crystallization process (18).

Local species of Calvatia, *C. lilacina* (puffball) gave antitumor principles in the sporophore as well as when cultivated under laboratory conditions using a modified Czapek's medium with 40% coconut water. It produced a yellowish white substance when purified by freezing and thawing followed by lyophilization. The substance obtained shows an inhibitory action *in vitro* on Ehrlich ascites tumor cell, following Miyamura's method (15).

In the case of a mushroom, *Psathyra umbonata*, which was grown in an artificial culture medium containing mineral salts and an enricher, the antibiotic substance was detected not only in the mycelial growth but also in the metabolite secreted by a mycelium into the liqiud medium. Other antibiotically active mushrooms which were successfully cultivated such as *P. cinnabarinus*, *Lentinus squarrusulus* and *C. lilacina* were found to pro-

Table 7. List of Outstanding Basidiomycete Species among the 587 Isolates (20)

Basidiomycete species	*Degree of inhibition of the test bacteria*						
	I	*II*	*III*	*UV*	*V*	*VI*	*VIII*
1. *Trametes* sp. 59157	+++	++	+	+	+	+	+++
2. *Psalliota argyrostica* Copeland 60225	++	+++	+++	+	++	+	++
3. *P. comtula* Fries 59137	+++	+++	+++	++	+++	—	++
4. *P. merilli* Copeland 59138	+++	++	+++	+	++	++	+
5. *P. subrufescens* 60232	+++	+++	+++	+	++	—	++
6. *Psathyra umbonata* 59141	+	+++	+++	+	—	—	—
7. *P. umbonata* 59142	+++	+	+++	+	+	+	+++
8. *Clithcybe candicans* Fries 62005, 62006	+++	+++	+	+	+	—	+++
9. *Copeland papilonaceae* (Bull.) Bres. 62010	+++	+++	+++	++	—	—	—
10. *Cortinarius violaceous* Fries 59037	+++	+++	++	+	—	—	+++
11. *Cortinarius* sp. 59038	+++	+++	+++	+	+	+	+++
12. *Galera tenera* Fries 59053	+++	—	+	+	—	—	+++
13. *Lepiota americana* Peck. 60113	+++	+++	++	++	+	+	+++
14. *L cepaestipes* Fries 59078	+++	+++	++	++	+	+	+++
15. *L. cepaestipes* Fries 59079	+++	+++	++	+	+	—	+++
16. *L. cepaestipes* Fries (3 strains)	+++	+++	+++	+	++	+	++
17. *L. cepaestipes* (2 strains)	+++	+++	+++	+	+	+	+++
18. *L. cepaestipes* 60129	++	+++	++	++	—	+	+++
19. *L. cepaestipes* 60130	+++	+++	++	+	—	—	—
20. *L. cepaestipes* 60125	—	+++	++	+	+	+	+++
21. *L. metulispora* Berk and Bres. 59086	+++	++	++	+	+	+++	++
22. *L. Lepiota* sp. 60143	++	+++	+++	—	++	+	++
23. *Pluteulus coprohilus* Peck. 59104	+++	+++	++	++	+	+	+++
24. *Polyporus cinnabarinus* (Lacq.) Fries 61053	+++	+++	++	+	+	+	+++
25. *P. sanguineus* Fries 61062	+++	+++	+	+	++	—	+++
26. *Polyporus* sp. 59111	+++	+++	+	+	+	—	+++
27. *Polyporus* sp. 59119, 52120	+++	++	++	++	+	+	++

Legend:

I — *M.p. aureus*
II — *M.p. aureus* (penicillin resistant)
III — *B. subtilis*
IV — *E. coli*
V — *Salmonella gallinarum*
VI — *Ps. aeruginosa*
VII — *Alkaligenes faecalis*

inhibition zone
+++ — 25 mm and above
++ — 27 — 24 mm
+ — 9 — 16 mm

duce the active substance both in their mycelium and in the culture medium (15).

Table 8 shows the local species cultured in artificial media for production of antibiotics.

A survey into the tumor inhibitory activity of the Basidiomycete samples using Ehrlich ascites tumor cell system gave interesting results. Table 9 gives a list of the active ones with strong inhibition shown by intensity of color and big inhibition zones.

An outcome of the screening for antibiotically active Basidiomycetes, a list of of some of the active ones with their local names is presented in Table 10.

Lower Fungi

A series of antifungal compounds is produced by a *Geotrichum* species (5) isolated as an air borne contaminant in copra. The antibiotics are produced in coconut water or defined medium and have been extracted from broth on an XAD-2 resin, eluted from resin with methanol, and chromatographed over a silica gel column, yielding two well separated antifungal components. Each of the two consists of a pair of antibiotics characterized by thin layer chromatography, mass spectrometry, and nuclear magnetic resonance, ultraviolet and infrared spectroscopy. Based on their characteristics and comparison with authentic samples. The present antifungal antibiotics are distinct from the azasteroidal antibiotics (A 25822) described earlier from Geotrichum flavobrunneum NRRL-3862 (J. antib. 28:95, 102, 112 (1975).

A polyene yellow antifungal antibiotic, novalichin (6) was isolated from a culture of *Paecilomyces fusisporus* ATCC 24148 grown in stationary culture on Czapek broth enriched with g/l — sucrose, 30.0, yeast extract — 2.0, sodium nitrate, 3.0, potassium chloride, 0.5, dipotassium phosphate 1.0, magnesium sulfate 0.5, ferrous sulfate 0.01, pH 4.5. From the mycelium, methanol extracted the compound with activity against *Candida albicans*. The antimicrobial spectrum (m.i.c.) in mcg/ml follows: *Ceratostomella paradoxa* 34, *Aspergillus oryzae* 68, *Saccharomyces cerevisiae* 68, *Candida abicans* 34. It was inactive against bacteria.

III. Algae

In their studies on the antimicrobic activity of 150 kinds of marine algae from Puerto Rico, Burkholder *et al.* (1) observed that 44 per cent were active against *S. aureus* and that the two species *Dictyopteris plagiogramma* and *Goniaulax tamarensis* which were usually found associated with the red tide water possessed both inhibitory and growth-promoting properties. Pratt

Table 8. Antibiotic Activity of Local Basidiomycetes Mycelium

Source	*Name of Basidiomycete*	*Culture media for propagation*	*Where activity was located*	*Antibiotic activity*	*Reference*
Quezon City Garden	*Psathyra umbonata*	mineral salts wood enricher (yeast extract)	mycelium and culture fluid	gram positive	16
Metro Manila	*Polyporus cinnabarinus*	mineral salts solution with rice bran	mycelium and culture fluid	gram positive	19
Metro Manila	*P. sanguineus*	mineral salts solution with rice bran	mycelium and culture fluid	gram positive	19
Quezon City	*Calvatia lilacina*	modified Czapek's solution with coconut water	mostly in mycelium	gram positive	16
Quezon City	*Lentinus squarussulus*	mineral salts with enricher (yeast extract)	mycelium and culture fluid	gram positive	16

Table 9. Antitumor Activity of Sporophore Extracts of Various Basidiomycetes on Ehrlich Ascites Tumor (cells (20).

Basidiomycetes	*No. of samples tested*	*Zone of Inhibition*	
		Degree	*Intensity*
Auriculariaceae			
Auricularia polytricha	1	++	*
Hydnaceae			
Hydnum adustum	1	++	*
Polyporaceae			
Boletinus sp.	1	+	*
Ganoderma mastoporum	1	+	*
Lenzites sp.	2	+	*
Polyporus gilvus	1	+	*
P. grammocephallus	1	++	*
P. cinnabarinus (Jacq.) Fries	1	++	**
P. sanguineus Fr.	1	+	*
Trametes corrugata (Pers.) Bres.	1	+	*
T. hirsuta (Wulf. ex. Fr.) Lloyd	2	++	**
Agaricaceae			
Clitocybe nebularis Batsch	1	++	*
Clitocybe sp. (Las Piñas)	1	+	*
Clitocybe sp. (Batangas)	1	++	**
Collybia confluens Pers.	2	++	*
C. deliciosa	1	+	*
C. dryophila Fries	3	++	**
Copelandia papilonacea (Bull.) Bres.	1	+	*
Coprinus cothurnatus God.	1	+	*
C. micaceus Fr.	1	+	*
Crepidotus mollis	1	+	*
Eccilia griseo-rubella Lasch	1	++	*
Entoloma sp.	1	+	*
Galera tenera Fr.	1	+	*
Galera sp.	1	+	*
Hebeloma crustuliniforme Fr.	1	++	*
Lepiota americana Peck.	1	++	*
L. cepaestipes	5	+++	**
Marasmius graminum Berk.	1	++	*
Naucoria pediades Fr.	1	++	*
N. platysperma	1	+	*
N. semiorbicularis Fr.	4	++	*
Omphalia reclines	1	+++	**
O. umbellifera	1	+	**
Omphalia sp.	1	++	*
Psathyra umbonata	2	+	*
Psalliota compestris var. *umbrina* Fr.	1	+++	**
P. perfuscus Copeland	3	+	*
P. comtula Fr.	1	+++	**
P. luzoniensis Graff	1	+	*
Schizophyllum alneum Linn.	2	+	**

Lycoperdaceae			
Bovista pila Berk and Curtis	1	+	*
Calvatia lilacina Fries	1	+++	**
Lycoperdon gemmatum Batsch	1	+	**
L. pyriforme	1	++	*

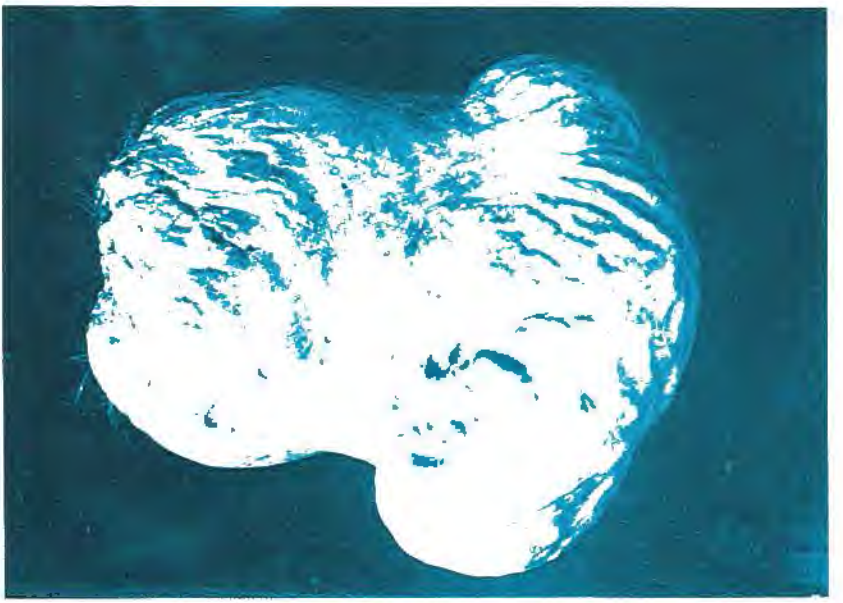
Legend:

Inhibition zone
+++ – 21-25 mm
++ – 16-20 mm
+ – 11-15 mm

Intensity
**** — dark blue zone**
*** — blue zone**

Table 10. Local Names of some Antibiotically Active Mushrooms

Scientific Name	*Common Name**	
Auricularia affinis (Leveille)	Taingang daga (Tag); dolongan sand kahoy (Panay)	Edible
Auricularia auricula-judae (Linnaeus)	Same as above	"
Auricularia polytricha (Montagne) Saccardo	Same as above	"
Calvatia lilacina (Fries)	Tombong (Tag.); parapara (Ilocano)	"
Clautriavia merulina (Phallus merulinus Berkeley)	Kabuteng may pandong (Tag.) oong ti uleg (Ilocano)	Poisonous
Clavaria crispa (Sulfen)	Oong nga repolio (Ilocano)	Edible
Calvaria stricta (Persoon)	Kabuteng bulaklak ng bato (Tag.)	"
Collybia albuminosa (Bresadola) Petch	Kabuteng punso or kabuteng pusngo (ag.) oong ti bunton (Ilocano); oong na pangol (Pangasinan); payung-palungan kulog (Pampanga)	
Colybia distorta (Fries) Gillet	Kabuteng pilipit (Tag.)	"
Copelandia papilonacea (Bulliard) Bresadola	Kabuteng taing kalabaw (Tag.)	Not tested
Coprinus comatus (Fries)	Kabuteng Kampanilla (Tag.)	Edible
Cortinarius collisteus (Fries)	Kabuteng Kalauangin (Tag.)	Suspicious
Cyathus striatus (Hoffmann)	Pugad ng ibon (Tag.)	Not Edible
Daedalea flavida (Leveille)	Kabuteng kapis (Tag.)	" "
Daldinia concentrica (Bolton) Cestadi and de Notaris	Kabuteng matigas at mabilog (Tag.)	Not specified
Galera tenera (Fries)	Kabuteng payat ang tangkay (Tag.)	Not tested



Calvatia lilacina, known as tumbong in Pilipino is a sample of mushroom. It has antibiotic and antitumor activity.

Table 10 (continued)

Scientific Name	*Common Name**	
Lentinus elmerianus (Lloyd)	Kulatkulat bundoc (Tag.)	Edible
Lentinus exilis (Klotzsch)	Kulatkulat kawayan (Tag.)	"
Lentinus squarrusulus (Berkeley and Curtis)	Kulatkulat na may kaliskis (Tag.)	"
Lepiota americana (Peck)	Kabuteng mamulamula (Tag.)	"
Lepiota cepaestipes (Fries)	Kabuteng may singsing (Tag.)	Poisonous
Lepiota chlorospora (Copeland) (L. Morgani Peck)	Payong ahas (Tag.); oong ti takki noang (Ilocano); oong na tai	"
Lepiota cristata (Fries)	Kabuteng tigre (Tag.)	Suspicious
Lepiota deundata (Rabenhorst)	Kabuteng kolor azufre (Tag.)	Poisonous
Lepiota lilacea (Bresadola)	Kabuteng lila (Tag.)	Not tested
Maramius haematocephalus (Montagne)	Kabuteng mukang balat (Tag.)	Edible
Naucoria semiorbicularis (Fries)	Kabuteng kinalauang (Tag.)	Not specified
Panaeolus campanulatus (Linnaeus)	Kabuteng kampana (Tag.)	Not tested
Panus rudis (Fries)	Kulatkulat morado (Tag.)	Not specified

Table 10 (continued)

Scientific Name	*Common Name**	
Pleurotus canus (Quelet)	Alitaptap (Tag.); anandap (Ilokano)	Edible
Pleurotus porrigens (Persoon)	Alitaptap (Tag.); anandap (Ilokano)	"
Psalliota campestris (Fries)	Kabuteng parang na puti (Tag.)	Edible
Psalliota campestris (Linnaeus) var. Umbrina (Fries)	Oong ya balit (Pangasinan)	"
Psalliota cotula (Fries)	Payung-payungan malagu (Pampangan)	"
Psalliota merrilli (Copeland)	Kabuteng parang na bulik (Tag.)	"
Psalliota perfuscus (Copeland)	Kabuteng parang na may sing-sing	"
Schizophyllum alneum (Linnaeus) (Schizophyllum commune) Schroet	Cudet (Ilokano); Kunas or sigdot (Tag.)	"
Volvaria esculenta (Bresadola)	Kabuteng ginikan or kabuteng saguing (Tag.); oong ti garami or oong ti saba (Ilokano); oong na puti or oong na dayami (Pangasinan)	

*The local names were taken from Mendoza "Philippine Mushrooms."

et al. (7) have shown that the unicelluar green alga, belonging to genus Chlorella, is the source of an antibiotic known as chlorellin, and that extracts of several species of marine algae inhibited the growth in vitro of one or more of the following bacteria: *S. aureus, (M.p. var. aureus), E. coli* and *P. aeruginosa.*

Other reports on the antibiotic activity of aqueous or organic solvent extracts of seaweeds include those of Kamimoto (2, 3) on the effects of extracts from seaweeds against the growth of some pathogenic organisms, acid fast bacteria and of Mauntner (7) on the antibiotic activity of *Rhodomela larix.*

Since algae abounds in our coastal waters, a survey of their antibiotic activities would increase our knowledge in their medicinal potentials.

Several species of marine algae collected from various places in the Philippines were screened for their antibiotic activity against test organisms commonly used for testing (11). Samples were washed, drained and ground to fine pieces. They were extracted with organic solvents and the dried extracts tested. Identification

of the species was kindly done by Dr. Paciente Cordero. A list of the species and their origin is given below:

From San Esteban, Ilokos Sur

001 *Sargassum enerve*
0021 *S. hemiphyllum*
S. fulvellum
0051 *Turbinaria trialata*
007 *Hormophyra triquetra*

From Pangasinan

002P *S. hemiphyllum*
004P *S. duplicatum*

From Matabungkay, Batangas

002B *S. hemiphyllum*
004B *S. duplicatum*
005B *Turbinaria trialata*

From Dumaguete City

006 *S. yendoi*
007 S. *crispifolium*

It can be seen from Table 11 that the ether and ethyl acetate extracts of algae shows the most inhibitory power. The sargassum species failed to inhibit *M. pyogenes* var. *aureus* 209 P, but inhibits *B. subtilis* and *B. cereus. H. triquetra* had very strong inhibition of *Myco* 607. *T. trialata* (IS) has strong activity on *M.p. aureus* 209 P and weak on *B. subtilis* and *Myco.* 607.

Ether extracts of samples showed inhibitory activity on the Gram positive test bacteria *B. subtilis.* Hexane, petroleum ether and aqueous extracts gave negative results. Methyl alcohol, ethyl alcohol, acetone extracts gave light inhibition while ethyl acetate extracts gave the strongest inhibition.

When tested on *Lymphocytic leukemia* P. 388, some samples such as *S. fulvellum* (I. Sur), *S. yendoi* (Dumaguete City), *S. crispifolium* (Dumaguete City) and *T. trialata* (I. Sur) showed positive indication of antitumor activity. However the results need confirmation.

Chlorella pyrenoidosa was cultivated in the presence of sunlight and CO_2 in a mineral salts solution. When sufficient growth has taken place. it was centrifuged to get the algal cells. Ether extracts of the cells produced slight inhibitory activity on the gram positive test bacteria.

IV. Lichens

Lichens are dual organisms composed of algae and fungi growing together, hence lichens cannot exist without algae and fungi (5). Lichens are not plants at all, but only associations of two plants (fungus and algae) growing together and botanists have always given them generic and specific names comparable to those of nonsymbiotic plants such as fungi, mosses or ferns. They held a

high place in the pharmacopoeia of medical doctors during the middle ages.

Foreign lichen species have been reported to be used as food and medicine as well as the dye and perfumery industries. As early as 1700 the lichens *Cetraria islandica*, *Lobaria pulmonaria*, and the *Usnea* species have been known to be used extensively as demulcents, tonics, febrifuges, purgatives and antitubercular drugs. In 1896 Chiba observed that tinctures prepared from several species of Usnea were beneficial in the treatment of Lymphadenitis tuberculosa colli (4a).

The antibiotic activities of foreign lichens have been reported by Stoll *et al.* (23), Burkholder and Evans, (3), Litvinov and Rassadina (10), Asahina and his associates, Kutami, Borkowski *et al.* (2), Liu *et al.* (9) and of Philippine lichens by Sevilla-Santos *et al.* (16). In general it was found that the gram positive bacteria and Mycobacteria were inhibited by the lichen extracts. Asahina and N. Kutami established the presence of lichen acids in native lichens in Japan. Seshadri (14) conducted a chemical investigation of Indian lichens and isolated compounds belonging to the fatty acid, depside, depsidone, pulvinic acid, anthraquinone, phenanthrene —

Sargassum duplicatum, is a sample of marine algae from Batangas. It has activity against some bacteria and Ehrlichs ascites tumor cells.

Table 11. Antibiotic Activity of Algal Extracts on Eight Test Organisms

Samples / *Samples*	*Acetone*				*Chloroform*	*Benzene*	*Ethanol*			*Ether*			*Ethyl acetate*					
	Bs	*Bc*	*Ec*	*Sc*	*Ec*	*Ec*	*Bc*	*Ps*	*Ca*	*Bs*	*Bc*	*Ps*	*Ma*	*Bs*	*Bc*	*Ec*	*Mt*	*Ca*
Sargassum enerve (IS)		—											—	+		+	+	
S. hemiphyllum (IS)		—								+	+	+	—	++	+	+		
S. hemiphyllum (B)		—								+								
S. hemiphullum (P)	—		+	—	++	+++	—					+		+				
S. fulvellum (IS)		—								+				++				
S. duplicatum (P)										+			—					
S. duplicatum (B)												+		+	+			
Turbinaria trialata (IS)													+++	+			+	
T. trialata (B)							+						+	+	+	+		
S. yendoi (D)													—	+				
Hormophyra triquetra (IS)													+	+			+++	+
S. crispifolium (D)									+				—	+	+			
Control							—		—	—	—			—	—		—	—

Legend:

Source:
- IS — Ilocos Sur
- B — Batangas
- P — Pangasinan

Test organism:

Ma:	*M.p.* var. *aureus* 209 P	Ps:	*P. aeruginosa*
Bs:	*B. subtilis*	Mt:	*Myco. 607*
Bc:	*B. cereus* var. *mycoides*	Sc:	*S. cerevisae*
Ec:	*E. coli*	Ca:	*C. albicans*

Degree of Inhibition:
- +++: 21-25 mm
- ++: 16-20 mm
- +: 12-15 mm
- —: 11 below

quinone, xanthone and diphenylene oxide groups. Dahl and Hale (5) also isolated some of these types of compounds and utilized them in lichen systematics for distinguishing chemical strains.

The antibiotic property of the chemical components of lichens were studied by Stoll *et al.* (23), Burkholder and Evans (3), Borkowski *et at.* (2) and Liu *et al.* (9) and they found that the lichen substances salazinic acid, stictic acid, usnic acid, prototive bacteria and mycobacteria but were inactive against the gram negative forms.

Usnic acid derivatives were tried by Takai *et al.* (25) on cultured L1210 cells and *in vivo* against Lewis lung tumor and Murine P 388 leukemia. None of the derivatives was more potent than Usnic acid in the *in vitro* assay. It was active against Lewis lung tumor and had some activity on the P 388 leukemia test system. The study on polysaccharide from *Gyrophora esculenta* indicated antitumor activity (11).

Interest in Philippine lichens dated as early as 1836 when Charles Gaudichaud, a French botanist, visited Manila and collected five lichens. This was followed by a systematic study of these lower plants by Wainio (26-30) followed by Herre (6-8). Quisumbing (12) reported a medicinal lichen *Usnea philippina.*

A study of the antibiotic activity of lichens which abound in the Philippines may pave the way for the exploration of the untapped lichen flora as sources of medicines which may be of far reaching significance in our country's drug shortage.

Lichen samples were collected from barks of trees, from soils, rocks and stone walls in Quezon City, Baguio City, Cavite, Rizal, Batangas, Mt. Mayon, Albay Province. Their antibiotic activity against 12 test microorganisms composed of 3 gram positive bacteria, 4 gram negative, 1 acid fast, 2 yeasts and 2 filamentous fungi was determined. Results obtained show that 30 extracts from 33 lichens inhibited at least one of the test organisms (20).

Table 12 shows 14 very active ones with strong activities on the gram positive bacteria and acid fast bacteria and light activity on the test fungi. The most active lichen extract against the gram positive bacteria are: *Lecanora subfusca, L. varia, Lepraria chlorina, Ramalina farinacea* and *Usnea montagnei,* while against *Myco.* 607 are *Crocynia membranacea, R. farinacea* and *U. montagnei.*

To determine the chemical constituents of the lichens, thin layer chromatography of these extracts was conducted (15). Lichen substances salazinic acid, stictic, acid, Usnic acid, protocetraric acid, barbatic, zeorin, atranorin, lecanoric acid, homosekikaic acid, lecanoric acid were run with the compounds obtained from the lichens. Table 13 shows the chemical consti-

Table 12. Showing the degree of antibiotic activity of lichen extracts (19)

Name of Lichen	*Degree of response to test bacteria*			
	Number of Samples Tested	*Gram positive bacteria (all)*	*Mycobacterium 607*	*Fungi*
Crocynia membranacea	2	++	+++	+
Lecanora subfusca	3	+++	++	+
L. sp. ARL 69	1	++	++	+
L. varia (Ehrl.) ARL 97	1	+++	—	++
Lepraria chlorina ARL 34	1	+++	++	—
Parmelia dactylifera Vainio ARL 75	1	++	++	++
P. zollingeri	1	++	—	—
Physcia albicans (Pers.) Thoms.	2	+	—	+
P. picta (sw.) Nyl. ARL-60	1	++	—	++
Physcia sp.	2	++	—	—
Ramalina farinacea (L.) Ach.	1	+++	+++	+
Ramalina sp.	3	++	++	—
Stereocaulon sp.	2	++	++	—
Usnea montagnei	1	+++	+++	+

Legend: **Inhibition zone**

+++ – 25 — 32 mm
++ – 17 — 24 mm
+ – 9 — 16 mm
– – 9 below

tuents and it can be seen that most of the lichens tested especially the *Usnea* species contain usnic acid, a potent antitubercular substance.

Polysaccharides were isolated by hot aqueous extraction of two lichens (*R. farinacea* and *U. montagnei*) (22) and when tested on P 388 and Leukemia L1210 gave promising results.

Since some of our native lichens contain polysaccharides and usnic acid, it might be worthwhile to conduct further investigations so that these two potentials of antineoplastic action could be exploited.

An attempt to identify the active component and verify the antibiotic activity led to the chemical study of some active species namely: *Usnea intercalaris (U. montagnei) U. elmeri, Physcia albicans, P. picta, Crocynia membranecea* and *Ramalina farinacea* (16, 22, 30).

Table 13. Showing chemical constituents of Philippine lichens detected by thin layer chromatography (15)

Name of Lichen	*Lichen substances*								
	Usnic acid	*Zeorin*	*atranorin*	*barbatic acid*	*lecanoric acid*	*salazinic acid*	*stictic acid*	*protocetraric acid*	*homsekikaic acid*
Usnea elmeri Heree	*	*				*	*		
U. flexilies (Stirt)	*					*	*	*	
U. hossie Vain	*			*		*			
U. intercalaris Kremp	*					*		*	
U. squarrosa Vain	*			*		*	*	*	
Physcia albicans (Pers.) Thoms.		*	*						
Parmelia cetrata Ach.			*		*				
P. zollengeri Hepp.	*		*						
Crocynia membranacea (Dicks) Zahler	*	*							
Ramalina farinacea (L.)	*								*
Stereocaulon sp.			*						

*shows the presence of chemical constituents

Six lichens were studied in detail. This included the isolation and identification of the constituents and determination of the antibiotic activities.

Thallus of lichens, *Usnea montagnei* or *Ramalina farinacea*, is known as lumot niyog in Pilipino. They have antibacterial and antitubercular activities.

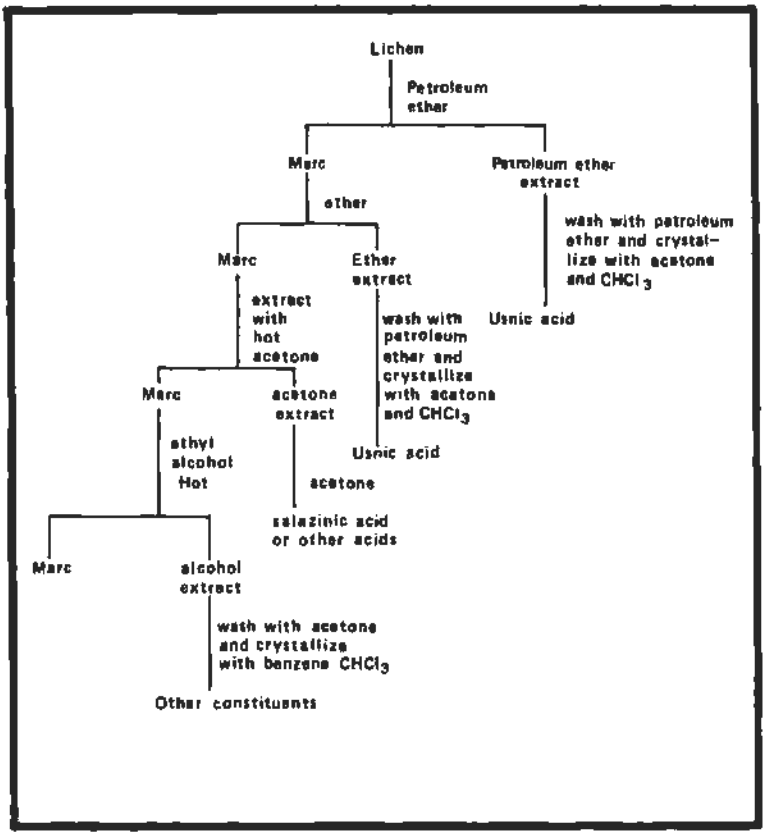


Figure 5. Schematic diagram showing isolation of chemical constituents of lichens.

A method for the isolation is given in the schematic diagram (Fig. 5).

Table 14 gives the chemical constituents which were obtained from the lichens studied with their physical and chemical properties as well as antibiotic activities.

Usnic acid, yellow, prismatic crystals melting at 198-210°C was obtained from *U. montagnei* (1.17%), *U. elmeri* (traces) and *C. membranacea* (0.57-0.96%).

The minimum inhibitory concentration for *M.p.* var. *aureus*, *M. aureus* (penicillin resistant), *B. subtilis* and *Myco.* 607 in μg/ml was 2, 3, 2 and 3 respectively.

Salazinic acid a white, fluffy silk-like, shiny needles, m.p. 240-260°C was isolated from *U. montagnei* (4.08%) and was inhibitory only to *M. aureus* (penicillin resistant) at 1 mg./ml.

Atranorin, colorless, prismatic crystals m.p. 196-197°C was obtained from *P. albicans* (2.3%) and *P. picta* (0.1%). The mini-

mum inhibitory concentration in μg/ml for *M.p.* var *aureus*, *M.p. aureus* (penicillin resistant) and *B. subtilis* was 0.133, 0.05 and 0.333 respectively.

Conclusion

I have endeavored to put before you some of what I consider to be important in my research efforts.I could of course have told you a great deal more and kept you here several hours while doing so, but there are more detailed documents setting out these matters for anyone interested.

In closing, I should like to point that antibiotic research is a very expensive endeavor. One should have sufficient and available funds, in order to keep experienced and efficient research workers and provide them with income, security and facilities. I find it rather difficult to find good jobs for admirable, young research workers in my own laboratory. They are the next generation of scientists and I feel that promising ones should be given all the encouragement and security.

This research on antibiotics started at the Bureau of Research and Laboratories, Department of Health under the sponsorship and guidance of Dr. Walfrido de Leon Sr. This was then transferred to the National Institute of Science and Technology, NSDB thru the leadership of Dr. Paulino Garcia (Chairman), Dr. C. Manuel and Dr. J. Velasco, Commissioners, where it stayed for some time. At present, I am continuing this research at the Research Center of the University of Santo Tomas with the encouragement of Rev. Fr. Ciriaco Pedrosa, O.P. Almost all the while the research has been largely and generously funded by the National Research Council of the Philippines. To them and to the NRCP, I am deeply grateful.

Perhaps, I might end on a personal note. I have been extraordinary well accorded by my scientific colleagues who sometimes over estimated my worth as a scientist. I am grateful to them for having done so, but I would like to place on record that my work could not have been accomplished without the joint effort of a number of people. To those who have worked with me in the laboratory and to those who collaborated with me in my research activities and who provided me with the inspiration and encouragement, I am also very grateful. If I have not mentioned them all by name it is because I have done so elsewhere and it would take up too much time.

Table 14. Chemical constituents of some Philippine lichens

Chemical	*Lichen source*	*yield %*	*Physical and chemical properties*				**Minimum *inhibitory concentration* µg/ml**
				Melting point °C	*optical rotation*	*λ max mu*	
Usnic acid	*Usnea montagnie* *U. elmeri* *Crocynia membranacea* *Ramalina farinacea*	1.17 traces 0.57— 0.96	yellow prismatic crystals	202-203 198-201 208-210	$[\alpha]_D^{29}$ 494°	285,235	MA — 2.0 MAR — 3.0 BS — 2.0 M 607 — 3.0
Salazinic	*U. montagnie* *U. elmeri*	4.08 traces	white fluffy silk-like shiny needles	240 (brown) 260 (dec.)		250,312	MAR — 1000
Stictic acid	*U. elmeri*	0.52	white needles	268-270		313,237	n.d.
Zeorin	*C. membranacea* *Physcia albicans*	0.39— 6.64	colorless hexagonal crystals	218-246 230-232 245-247 225-227	$[\alpha]_0^{31}$ + 64° (C_1 $CHC1_3$	absorp tion no bands	no activity
Atranorin	*P. albicans* *P. picta*	2.3 0.1	colorless prismatic rods	1976-197	optically inactive	252 log E 4.7	MA — 0.133 MAR — 0.05 BS — 0.333
Atranorin— chloratrano-rin mixture	*P. picta*	1.6	crystalline			n.d.	

Legend:

MA — *Micrococcus pyogenes aureus* 209 P
MAR — *Micrococcus pyogenes aureus* (penicillin resistant)
BS — *Bacillus subtilis*
M 607 — *Mycobacterium* 607
n.d. — not

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DISCUSSION ON THE ANTIBIOTIC AND ANTITUMOR ACTIVITIES OF SELECTED PHILIPPINE THALLOPHYTES

Flordeliz R. Uyenco, Ph.D., Discussant

The paper of Dr. Patrocinio Sevilla-Santos on the antibiotic and anti-tumor activities of selected Thallophytes is to be commended for its comprehensive exploration into the potential of our lower plants for such activities. Nowhere in the annals of Philippine scientific investigations has a study like this been as thorough and encompassing. For these, Dr. Santos deserves to be congratulated.

The potential sources of antibacterial and anti-fungal drugs from Philippine seaweeds cannot be overemphasized. The archipelago teems with macroscopic green, brown and red algae which can be tapped for such purposes. *Digenea simplex*, a common red alga encountered in waters around Luzon, has been known to be an effective vermifuge. Its anti-viral property has been reported quite recently. Effective against the Influenza B and mumps viruses, this plant can be included among those in the list of economically important ones. The studies of a group of investigators headed by Prof. Leticia Angeles of the U.P. College of Medicine, have brought out the report that *Sargassum natans*, one of our common brown algae, possesses the anti-bacterial property against *Salmonella galinarum*, a parasite of poultry. *Phaeocystis pouchetii*, another brown alga, produces acrylic acid, an antibacterial compound.

The planktonic dinoflagellate, *Prymnesium parvum* has anticancer properties in a phospholipase-like toxin known as prymnesin. Macroscopic algae lend advantage in the production of drugs with such specific properties largely because of their abundance as natural resource in our waters. *Sargassum* can be farmed, thus assuring ample supply for extraction of drugs.

The Actinomycetes, or mold-like fungi, have long been known to produce antibiotic metabolites. *Streptomycin* is a household word. The biological implications of polyene macrolide-sterol interaction have contributed to our understanding of the mechanisms by which Actinomycetes affect sterol-steroid metabolism in animals. The polyene macrolide of Nystatin, for instance, is capable of acting on cell membranes of fungi and so can control systematic and topical fungal infections. The fungi

bind to this antibiotic in a sterol-containing site. Since bacteria do not contain sterols, they are insensitive to Nystatin.

Lichens have been shown by our speaker to effectively inhibit the TB organism and tumor cells. However, because of the very slow growth of lichens (about 1 cm. increase in diameter a year or 1 inch in length for fruticose forms) a massive program of mass production of antibacterial and anti-tumor substances from these plants, available at very low supply, becomes seemingly impossible and expensive.

Calvacin, an anti-tumor compound present in the basidiomycete, *Calvatia*, has been successfully isolated and tested by Dr. Santos from a local species. A similar compound has been extracted in the U.S. and tested on patients in a hospital in Texas. The results are encouraging. We should not lose time in putting up our own antibiotic plant because we have the resources right here.

Studies on the biochemistry and pharmacology of antibacterial agents would naturally follow the results of Dr. Santos' screening program. The biochemical basis of action, e.g., the action of these compounds at one particular site of metabolism and the mechanisms affected by antibiotics and anti-tumor substances should be investigated if only to determine the possible emergence of drug resistance. Such studies would be useful in dealing with the routes of administration of the compounds to man and animals, the ways in which the drugs are treated in the body, including absorption and distribution, metabolism and excretion, toxicity and interactions.

The mechanisms involved in antibiotics have been fully established although much remains to be done for our local isolates and screened microorganisms. Inhibitors of peptidoglycan, nucleic acid and protein syntheses are known but have these been determined for our own microflora?

Lydia M. Joson, Ph.D.

It is my great honor and pleasure that I have been asked to give comments on the life time work of Dr. Patrocinio Sevilla-Santos. I was once upon a time one of those youths she mentioned in her introduction whom she had inspired and to whom she had passed her knowledge of antibiotic research. It has been my good fortune that I had worked directly with two of the most-respected and prolific scientists of our time, Dr. Alfredo C. Santos and Dr. Patrocinio Sevilla-Santos. Both of them together with Mrs. Luz Baens-Arcega have influenced my scientific inclination and career. To them my deep appreciation and gratitude.

Dr. Santos' work on the antibiotic and anti-tumor activities of the thallophytic is quite embracing. She and her co-workers have done surveys of antimicrobial and antitumor activities of streptomycetes from soil, indigenous mushrooms, lichens and marine algae. Steptomycetes capable of producing actinomycins, tetracyclines and penicillins from fungi had been isolated. The potentially good ones were further studied for the production of the active principles using locally-available raw materials, followed by the isolation and characterization of the desired products. The antibiotic yields, however, have been rather low. To bring these yields to industrial scale, strain improvement programs must be instituted. And this is where R and D in antibiotic research in the Philippines should be emphasized as well as the search for new antifugal antibiotics.

The world-wide search for new antibiotics is still being actively pursued, for there are needs for new substances that are active against resistant organisms to known antibiotics; substances active against targets not covered by known antibiotics; also for new substances with better pharmacological features than known antibiotics.

It is hoped that an integrated approach to R and D in antibiotics would bring about the discovery of new antibiotics or the development of technology that will bring about the establishment of an antibiotic plant in the Philippines — a long cherished dream of Dr. Santos.