cooler conditions since the Eocene, *Paulownia* migrated southward with other temperate deciduous plants until by the middle Tertiary it had reached middle latitudes. Since then it has died out on all continents except Asia, possibly as a result of late Cenozoic glaciation and of barriers to migration to more southern latitudes.

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THE EFFECTS OF SELECTED ANTIBIOTICS ON PURE CULTURES OF ALGAE[1]

EDWARD O. HUNTER, JR.[2] AND ILDA MCVEIGH

ABSTRACT

HUNTER, E. O., JR., and ILDA MCVEIGH. (Vanderbilt U., Nashville, Tenn.) The effects of selected antibiotics on pure cultures of algae. Amer. Jour. Bot. 48(2): 179–185. 1961.—A determination was made of the effectiveness of various concentrations of actidione, nystatin, amphotericin-A, anisomycin, and sulfocidin, antibiotics active primarily against fungi, in inhibiting representative species of Myxophyceae, Chlorophyceae, Bacillariophyceae, Xanthophyceae, and Euglenophyceae. Similar investigations were made using polymyxin-B sulfate and bacitracin, antibiotics inhibitory to certain groups of bacteria. Concentrations of 1, 2, 20, 50, 100, and 200 p.p.m. were used. Concentrations of 200 p.p.m. or less of anisomycin, nystatin and actidione had little or no detectable effect on the Myxophyceae but were toxic to members of the Chlorophyceae and the Bacillariophyceae. Thus, these 3 antibiotics are of potential value in eliminating green algal and diatom contaminants from cultures of blue-green algae. Since bacitracin was found to be inhibitory to members of the Myxophyceae at concentrations not toxic to representatives of the other groups tested, it may prove useful in eradicating blue-green algae from cultures of other forms. Microscopic examinations indicated that the cells of cultures of species belonging to the Chlorophyceae, the Myxophyceae, and the Euglenophyceae, when exposed to antibiotics to which they were sensitive, usually underwent lysis, while those of species of Xanthophyceae and of Bacillariophyceae generally showed a loss in pigmentation.

THE POTENTIAL VALUE of antibiotics in relation to their action on microscopic forms of plant life has not been fully determined. For example, few investigations have been made to ascertain the effects of antibiotics on algae. Provasoli et al. (1948) were among the first investigators to show interest in the use of antibiotics to obtain pure cultures of algae. They reported that some strains of *Euglena gracilis* underwent a permanent loss of chlorophyll as a result of exposure to certain concentrations of dihydrostreptomycin. However, the algae continued to grow when suitable energy sources were supplied. In 1951, Provasoli et al. made determinations of the concentrations of penicillin, streptomycin, chlorotetracycline, oxytetracycline, chloramphenicol, bacitracin, and polymyxin tolerated by representative species of algae and protozoa. They found that the use of appropriate combinations of the antibiotics resulted in the elimination of both gram-positive and gram-negative bacterial contaminants from the algal and protozoan cultures. Successful attempts to obtain bacteria-free cultures of algae were reported also by Pappas

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and Hoffman (1952). They succeeded in obtaining bacteria-free cultures of *Euglena gracilis* by the use of combinations of penicillin, dihydrostreptomycin, and chlorotetracycline. Bacitracin and sulfadiazine were too toxic to the *Euglena* to be useful.

From 1952 to 1958 there was a paucity of literature concerning the use of antibiotics as a means of obtaining pure cultures of algae. However, during this period, several articles concerning the potential use of antibiotics as algicides in the purification of water supplies were published. Foter et al. (1953) evaluated the antialgal properties of polymyxin-B, chlorotetracycline, bacitracin, chloramphenicol, neomycin, penicillin, streptomycin, gliotoxin, and oxytetracycline on selected species of diatoms and blue-green and green algae. Two years later, the results of an investigation of the inhibitory effects of numerous inorganic and organic compounds, including 5 antibiotics, on representatives of the same groups of algae were reported by Palmer and Maloney (1955). Their results showed that actidione at concentrations resulting in complete inhibition of green algae and diatoms had little or no effect on blue-green algae. As previously reported by Foter et al., streptomycin sulfate was particularly inhibitory to blue-green algae and diatoms but had little effect on green algae.

Interest in the use of antibiotics as a means of obtaining pure cultures of algae was revived in 1958. Zehnder and Hughes (1958) reported that actidione, at concentrations of 50 p.p.m. or less, inhibited the growth of members of the Chlorophyceae, Xanthophyceae and Bacillariophyceae. Much higher concentrations had no inhibitory effect on the development of the Myxophyceae. In 1959, Galloway and Krauss reported an investigation of the differential action of chemical agents on certain algae, bacteria, and fungi. Polymyxin-B sulfate was found to have a high degree of selectivity, inhibiting the bacteria tested at concentrations which had little or no effect on *Scenedesmus obliquus* and *Chlorella vulgaris*. The investigation reported here was undertaken in order to determine the effects of selected antibiotics, primarily agents inhibitory to fungi, on representative members of the Myxophyceae, the Chlorophyceae, the Bacillariophyceae, the Euglenophyceae, and the Xanthophyceae in pure cultures and to observe the morphological changes in the algae caused by the antibiotics.

MATERIALS AND METHODS.—Bacteria-free stock cultures of algae were obtained from Dr. Richard C. Starr of Indiana University. The species and strains investigated are listed in table 1. Also indicated in the table are the media used in the determinations of the effects of the antibiotics and for maintenance of stock cultures of each of the strains.

Anisomycin, bacitracin, and polymyxin-B sulfate were obtained from the Chas. Pfizer and Co., Inc.; actidione from the Upjohn Company; amphotericin-A and nystatin from E. R. Squibb Chemical Company Stock solutions of polymyxin-B sulfate and of bacitracin were prepared by adding sterile, Pyrex-distilled water directly to the vials containing the antibiotic in a sterile powder form. A sterile suspension of nystatin, which is not soluble in water, was obtained in a similar manner. Amphotericin-A was dissolved in a mixture of 7 parts of methanol to 3 parts of 1.0 N NaOH and sterilized by passage through a sterile millipore filter. Anisomycin, sulfocidin, and actidione also were obtained in unsterile condition. Aqueous suspensions or solutions of these were sterilized by steam under pressure (121.5°C. for 15 min.). The desired concentrations of each antibiotic were prepared by making proper dilutions of each stock suspension or solution with sterile distilled water. The concentrations of the antibiotics to which the algae were exposed were 1.0, 2.0, 20.0, 50.0, 100.0, and 200.0 p.p.m. Freshly prepared stock suspensions or solutions of the antibiotics were used for each experiment.

Tests were performed in 150 × 16-mm. test tubes containing 9.0 ml. of the appropriate basal medium made to 0.9 final volume. To each tube of media, after sterilization, was added aseptically 1.0 ml. of the appropriate concentration of the antibiotic to be tested. To the tubes serving as controls, 1.0 ml. of sterile distilled water was added in place of the antibiotic solution. Special precautions were taken to cool the sterilized agar media to 45°C. before the addition of heat-labile antibiotics, and then, after agitation, to insure uniform distribution of the antibiotic throughout the medium, the tubes were partially immersed in ice water and slanted immediately. Each test was performed in triplicate Since polymyxin-B sulfate does not diffuse readily in agar media (Herman, 1954–1955) all tests in which it was used were made in liquid media.

Actively growing stock cultures, approximately 1 wk. old, were used as the source of inocula. The broth cultures were agitated to suspend the cells uniformly, and then by means of a 1.0-ml. pipette, 1 drop was added aseptically to each tube of liquid test medium. Inoculations of agar slants containing the antibiotics were made by streaking cells directly from a young stock culture onto each agar slant, exercising care to use a uniform amount of inoculum. All cultures were incubated at 24°C. and illuminated 12 hr. daily at a light intensity of 200 ft.-c. Stock and experimental cultures grown in liquid media were shaken thoroughly once each day.

Macroscopic and microscopic examinations were made of the test cultures after 1, 2, and 3 wk. of incubation. For these examinations, mounts were made by placing a loopful of the culture to be observed in a drop of Haupt's adhesive (Johansen, 1940) on a clean slide. The mixture was spread, allowed to dry, and then examined under oil immersion (960×). Motility studies were made by placing a loopful of culture on a clean glass slide and observing under low magnification (100×). At the termination of each experiment, in order

TABLE 1. *Concentration in p.p.m. of antibiotics causing partial or complete inhibition of the growth of certain algae during 1, 2, and 3 weeks of incubation. Concentration of 1.0, 2.0, 20.0, 50.0, 100.0, and 200.0 p.p.m. were used*

ORGANISM	MEDIA [a]	Actidione 1	2	3	Amphotericin A 1	2	3	Anisomycin 1	2	3	Nystatin 1	2	3	Bacitracin 1	2	3	Polymyxin B 1	2	3
MYXOPHYCEAE:																			
Nostoc sp., 389	Soil Extract Agar										No inhibition								
Fremyella diplosiphon, 481	Cyanophycean Agar										No inhibition								
Anabaena cylindrica, 629	"	No inhibition			No inhibition			No inhibition			No inhibition			2P 20C	2P 20C	1P 2C	No inhibition		
Phormidium sp 485	Proteose Broth	No inhibition			No inhibition			No inhibition			50P 200C	100P 200C	100P 200C	2C	2C	2C	2P 20C	20C	20C
CHLOROPHYCEAE:																			
Haematococcus lacustris, 294	Proteose Agar	1C	1C	1C	....	1P 2C	2C	20P 100C	20P 100C	50C	50P 200C	100P 200C	100P 200C	No inhibition			1C	1C	1C
Chlamydomonas agloeoformis, 231	Proteose Broth	2P 50C	2P 50C	2P 50C	No inhibition			20P 100C	20P 100C	50P 100C	20C	20C	20C	No inhibition			1P 50C	1P 50C	1P 20C
Ankistrodesmus falcatus, 188	Proteose Agar										2P 20C	2P 20C	2P 20C						
Hormidium sp., 623	Soil Extract Agar										1C	1C	1C						
Chlorococcum minutum, 117	Modified Bristol's Agar										1P 2C	2C	1C						
Scenedesmus obliquus, 393	Proteose Broth										No inhibition								
Chlorella pyrenoidosa, 252	"										50P 100C	50C	50C						
Stichococcus bacillaris, 314	"										100P 200C	100C	100C						
Goccomyxa elongata, St. 2,267	"										No inhibition								
XANTHOPHYCEAE:																			
Polydriella helvetica, 49	Proteose Broth	20P 200C	20P 50C	20P 50C	No inhibition			No inhibition			50P 200C	50P 200C	100P 200C	No inhibition			No inhibition		
BACILLARIOPHYCEAE:																			
Navicula pelliculosa, 674	Soil Extract Agar	2P 20C	2P 20C	2C	2P 100C	50C	50C	20P 100C	50P 100C	50P 100C	1C	1C	1C	50P 100C	50C	50C	No inhibition		
EUGLENOPHYCEAE:																			
Euglena gracilis "Z", 753	Euglena Broth	2P 100C	2P 100C	2P 100C	No inhibition			20P	20P	100P 200C	50P 100C	50C	50C	No inhibition			No inhibition		

C = Complete inhibition; P = Partial inhibition.

[a] Formula for modified Bristol's Agar medium was published by Bold (1949); those for the other media by Starr (1956, 1960).

to determine whether the effect of the antibiotic was lethal or static, a loopful of culture was transferred directly from tubes containing the lowest concentration of the antibiotic which apparently had caused complete inhibition of growth into tubes of the appropriate basal medium containing no antibiotic. After incubation for 7 days, observations were made to see whether or not growth had occurred.

RESULTS.—The concentrations of the antibiotics resulting in partial or complete inhibition of the various species of algae during 1, 2, and 3 wk. incubation are shown in table 1.

Actidione.—Neither of the species of Myxophyceae investigated was sensitive to actidione at concentrations of 200 p.p.m., whereas 100 p.p.m. or less resulted in complete inhibition of the representative species of the Chlorophyceae, Xanthophyceae, Bacillariophyceae, and Euglenophyceae. One p.p.m. of the antibiotic produced lysis of both vegetative and reproductive cells of *Haematococcus lacustris* in broth cultures during the first week of incubation. Fifty p.p.m. had a similar effect on *Chlamydomonas agloeoformis*, while 2–20 p.p.m. caused lysis of the zoospores. No further changes were noted in the cultures of these 2 species during the 3-wk. period of observation. Complete lysis of the cells of *Euglena gracilis* occurred within 7 days in broth containing 100 p.p.m. of the antibiotic. Concentrations of 2–50 p.p.m. caused partial inhibition accompanied by lysis of motile cells, but the cells in the palmelloid stage appeared normal. Examinations made after 3 wk. of incubation revealed no further changes in the morphology of the cells or in the amount of antibiotic resulting in complete inhibition. No evidence of lysis of cells of *Polydriella helvetica*, a xanthophycean species, or of the diatom, *Navicula pelliculosa*, was observed in cultures containing inhibitory concentrations of actidione. The cells, however, became colorless or nearly so. Within 1 wk., in the presence of 20–100 p.p.m., cells of *P. helvetica* contained less pigment than those of the control cultures, while in the presence of 200 p.p.m. the cells were colorless. At the end of 2 wk., cells in the presence of 50 p.p.m. of the antibiotic also were colorless. *N. pelliculosa* was somewhat more sensitive than *P. helvetica*. Within 2 wk., cells in cultures containing 20 p.p.m. were colorless. In 2 wk., those in the presence of 2 p.p.m. of the antibiotic contained less pigment than those in control cultures, and by the end of 3 wk., they also were colorless.

Amphotericin-A.—Concentrations of 1–200 p.p.m. of amphotericin-A produced detectable inhibitory effects on only 2 of the species tested, *Haematococcus lacustris* and *Navicula pelliculosa*. During the first week of incubation, *H. lacustris* failed to grow noticeably in either the control or test cultures, and no effects of the antibiotic were evident. However, after incubation for 2 wk., microscopic observations showed that 1 p.p.m. had produced lysis of the motile cells but had had no apparent effect on either the akinetes or aplanospores. On the other hand, a concentration of 2 p.p.m. had caused complete lysis of all cells, and, by the end of incubation for 3 wk., only scattered cell remnants were observed. The chloroplasts of cells of *Navicula pelliculosa*, the other species inhibited by amphotericin-A, were somewhat bleached after 7 days on media containing concentrations of 2–50 p.p.m. of the antibiotic, while in the presence of 100 p.p.m. only colorless cells were observed. After 2 wk. incubation, the cells in the presence of 50 p.p.m. of amphotericin also were colorless.

Anisomycin.—At the concentrations employed, anisomycin caused no detectable effects on the blue-green algae, *Anabaena cylindrica* and *Phormidium* sp., or on the yellow-green species *Polydriella helvetica*. However, both species of green algae, the euglenoid species, and the diatom were sensitive to anisomycin. In cultures containing 20–50 p.p.m. of the antibiotic both vegetative and reproductive cells of *Haematococcus lacustris* and *Chlamydomonas agloeoformis* settled to the bottom during the first week of incubation, while in higher concentrations lysis occurred. At the end of 2 wk. incubation, the same condition existed. However, by the end of 3 wk. the zoospores in cultures to which 20 p.p.m. of the antibiotic had been added were again active, and there was little evidence of sedimentation, while in the presence of 50 p.p.m. lysis of the cells of *H. lacustris* had occurred. Anisomycin, also, caused lysis of *Euglena gracilis*. Some of the motile cells of this species were lysed during the first week of incubation in media containing 20–200 p.p.m. Except in cultures containing 200 p.p.m. of anisomycin, the palmelloid cells appeared normal throughout the 3-wk. period of observation, but in those containing 200 p.p.m. lysis of both motile and palmelloid cells occurred. No lysis of the cells of *Navicula pelliculosa* was observed, but the chloroplasts were partially bleached in cultures containing 20 and 50 p.p.m. of anisomycin and completely bleached in those containing 100 p.p.m. within a week. Cells in cultures containing 20 p.p.m. appeared normal when observations were made after incubation for 2 and 3 wk., while in those containing 50 p.p.m. the chloroplasts were still partially bleached and with 100 p.p.m. completely bleached.

Nystatin.—On the basis of preliminary investigations, nystatin appeared to have an antialgal spectrum similar to that of actidione. In order to get a better indication of its possible usefulness in the removal of various algal contaminants from cultures of Myxophyceae, its effects on 9 additional species were determined. Of the 4 species of blue-green alga tested, only *Phormidium* sp. was sensitive to nystatin. Complete lysis of this species occurred within 1 wk. in media containing 200 p.p.m., while in cultures containing 50 and 100 p.p.m., several trichomes appeared to be partially lysed. *Anabaena cylindrica, Fremyella diplosiphon*, and *Nostoc* sp.

grew in the presence of 200 p.p.m., the highest concentration used, and, upon microscopic examination, the cells appeared normal. Of the 12 other species tested, which included 9 Chlorophyceae, and 1 each of Xanthophyceae, Bacillariophyceae, and Euglenophyceae, only *Scenedesmus obliquus* and *Coccomyxa elongata* were resistant to 200 p.p.m. of nystatin. Microscopic examination of cultures of *Chlamydomonas agloeoformis* indicated that complete lysis of both vegetative and reproductive cells had occurred in cultures containing 20 p.p.m. of the antibiotic within the first week of incubation. A similar condition was observed in cultures of *Ankistrodesmus falcatus* containing the same concentration of the antibiotic, while in those containing 2 p.p.m. partial inhibition was noted with lysis of some of the vegetative cells. No further changes were observed at later periods of examination. *Hormidium* sp. and *Chlorococcum minutum* were even more sensitive. A concentration of 1 p.p.m. resulted in complete lysis of all cells of the former species and of the zoospores of *C. minutum* within a week, while 2 p.p.m. produced lysis of all of the cells of the latter species. Evidently *C. minutum* recovered from the early inhibitory effects of 1 p.p.m. of nystatin, since normal zoospores as well as aplanospores were present in abundance when observations were made after incubation for 2 and 3 wk. Species of Chlorophyceae which were less sensitive to nystatin than those mentioned above were *Haematococcus lacustris, Stichococcus bacillaris,* and *Chlorella pyrenoidosa.* At concentrations of 200 p.p.m., all cells of *H. lacustris* underwent lysis during the first weeks of exposure, and, in cultures containing 50 and 100 p.p.m. of the antibiotic, some zoospores underwent lysis but the aplanospores appeared normal. When cultures containing 100 p.p.m. of nystatin were examined after incubation for 2 and 3 wk., normal aplanospores were observed but no motile cells. Cells of cultures of *S. bacillaris* were completely lysed by 200 p.p.m. of nystatin within a week and by 100 p.p.m. in 2 wk. Similar effects were produced in cultures of *C. pyrenoidosa* and *Euglena gracilis* by somewhat lower concentrations, 100 p.p.m. resulting in complete lysis within a week and 50 p.p.m. in 2 wk. Partial lysis of both species was evident at the end of the first week in cultures containing the lower concentration.

The inhibitory effect of nystatin on *Polydriella helvetica* and on *Navicula pelliculosa* differed from that just described for the other algae inhibited. Instead of causing lysis of the cells, it had a bleaching effect. After incubation for 1 and 2 wk., cells of *P. helvetica* in cultures containing 50–200 p.p.m. of the antibiotic appeared bleached in comparison to those of the control cultures. However, by the end of the 3-wk. period of observation, the cells of cultures to which 50 p.p.m. of nystatin had been added apparently had recovered since the plastids appeared normal in color. Partial bleaching was still evident in cultures containing 100 p.p.m. of the antibiotic, while in those containing 200 p.p.m. only colorless cells were observed. Cells of *N. pelliculosa* were completely bleached in cultures containing 1 p.p.m.

Sulfocidin.—Of the algae investigated, *Haematococcus lacustris* was the only species inhibited by the concentrations of sulfocidin used. Within a week, complete lysis of cells other than aplanospores occurred in cultures containing 50 p.p.m. of the compound, and the vegetative cells were lysed in concentrations as low as 1 p.p.m. The aplanospores, however, appeared normal in concentrations of 50 p.p.m., and by the end of 2 wk. had given rise to apparently normal cultures. Evidently, the sulfocidin in these cultures had deteriorated to the extent that it was no longer effective in lysing cells of this species or else the cells had become resistant to it. At concentrations of 100 p.p.m. and above, all cells were lysed.

Bacitracin.—The representatives of the Myxophyceae were found to be sensitive to low concentrations of bacitracin, an antibiotic primarily inhibitory to gram-positive bacteria. Microscopic examination indicated complete lysis of the trichomes of *Phormidium* sp. by concentrations as low as 2 p.p.m. during the first week of incubation. After the same period of exposure to 20 p.p.m. of the antibiotic, the cells of *Anabaena cylindrica* had become completely colorless, while in the presence of 2 p.p.m. they were only partially bleached, and in the presence of 1 p.p.m. they appeared normal. However, by the end of 3 wk., only colorless cells were observed in cultures containing 2 p.p.m. of bacitracin, and in those with 1 p.p.m. the cells were somewhat chlorotic. The only other species inhibited by bacitracin was the diatom, *Navicula pelliculosa.* A concentration of 50 p.p.m. resulted in partial bleaching of this species within a week and complete bleaching within 2 weeks.

Polymyxin-B sulfate.—Both species of Chlorophyceae and *Phormidium* sp., a blue-green alga, were inhibited by polymyxin-B sulfate, an antibiotic primarily effective against gram-negative bacteria. *Haematococcus lacustris* was extremely sensitive, lysis of all types of cells occurring during the first week of incubation on media containing 1 p.p.m. of polymyxin. This concentration also produced lysis of the vegetative cells of *Chlamydomonas agloeoformis* but not of akinetes. The least concentration resulting in lysis of all cells within a week was 50 p.p.m. By the end of 3 wk., cells of cultures containing 20 p.p.m. also had been lysed. Partial lysis of *Phormidium* sp., the only other species inhibited by polymyxin, had occurred in cultures containing 2 p.p.m. and complete lysis in those with 20 p.p.m. by the end of 1 wk. When examined at the end of 2 and 3 wk. incubation, cells of cultures to which 2 p.p.m. of polymyxin-B has been added appeared normal.

Viability tests.—In order to determine whether

viable cells were still present in cultures which appeared to have been completely inhibited, subcultures were made to media containing no antibiotic. After incubation for a week, no indication of growth was detected in any of the subcultures.

DISCUSSION.—Several articles concerning the effects of bacteriostatic and bactericidal antibiotics on algae and their use in freeing algal cultures of bacteria have been published (Provasoli et al., 1948, 1951). In contrast, the effects of few of the fungicidal and fungistatic antibiotics on the growth of algae have been determined. Before attempting to use such antibiotics as agents in eliminating fungal contaminants from cultures of algae, information concerning the sensitivities of the algae to them is desirable. Only those antibiotics which inhibit fungi at lower concentrations or in shorter periods of exposure than are required to inhibit the algae can be used successfully in freeing the algal cultures of the fungal contaminants. It was thought that some antibiotics might also have a selective inhibitory action on certain groups of algae and, therefore, prove useful in eliminating contaminants belonging to the sensitive groups from cultures of resistant forms.

The antifungal antibiotics, actidione, amphotericin-A, anisomycin, nystatin and sulfocidin, were, with one exception, not inhibitory to members of the Myxophyceae. As reported by Zehnder and Hughes (1958), and confirmed in this investigation, concentrations of actidione sufficient to cause complete inhibition of representative species of the Chlorophyceae, the Xanthophyceae, and the Bacillariophyceae produce no detectable effects on members of the Myxophyceae. In addition, actidione was inhibitory to the species of Euglenophyceae tested. Nystatin was found to have an antialgal spectrum very similar to that of actidione. Since previous work has shown that actidione (Whiffen, 1948, 1950; Ford and Leach, 1948) and nystatin (Frank et al., 1958–59) are very effective antifungal agents, and because of the differences in effects on various groups of algae as reported here and by Zehnder and Hughes (1958), the usefulness of these 2 antibiotics in freeing cultures of Myxophyceae, not only of fungal contaminants but also of algal contaminants belonging to other groups, seems promising. From the evidence available, actidione and nystatin offer more promise as aids in obtaining pure cultures of the Myxophyceae than do the other antifungal agents. These 2 antibiotics may also be of use in eliminating fungi from certain species of the other groups of algae, particularly those which are resistant to as much as 50–100 p.p.m. of the antibiotics, since lower concentrations are inhibitory to many yeasts and fungi. Sulfocidin is not likely to be of much use in freeing algal cultures of fungi since it deteriorates readily when in solution. Determinations of the effects of anisomycin and of amphotericin on a greater number of species of algae is desirable. The results of preliminary investigations indicate little or no toxicity of these 2 antibiotics for some of the species tested, and thus possibly they could be of use in obtaining pure cultures of such species.

Besides the antifungal antibiotics, 2 antibacterial agents were used. The inhibitory effects of polymyxin-B sulfate, active primarily on gram-negative bacteria, and of bacitracin, toxic primarily to gram-positive bacteria, on representative species of the various groups of algae were determined. In 1958, Nickell had reported that cells of certain flowering plants, for example, pole beans (*Phaseolus vulgaris*), Mexican yam (*Dioscorea composita*), avocado (*Persea americana*), and the century plant (*Agave toumeyana*), when grown in tissue cultures and stained according to a modification of Gram's method, were gram-negative. These cells were also found to be very sensitive to polymyxin-B but not inhibited by penicillin or by bacitracin except at very high concentrations. In the present investigation, attempts to stain the algae by modifications of Gram's staining procedure were, for the most part, unsuccessful, and the data are insufficient to determine whether there is a correlation between gram reaction of the algae and the sensitivity to antibiotics having a particular antibacterial spectrum. However, cells of young cultures of the species of Myxophyceae tested were gram-positive to gram-variable in reaction, and it is interesting to note that they were particularly sensitive to bacitracin.

Microscopic examinations revealed that cells of cultures of species of the Chlorophyceae, the Myxophyceae, and the Euglenophyceae, when exposed to antibiotics to which they are sensitive, usually undergo lysis, while those of the Xanthophyceae and of the Bacillariophyceae, forms in which silicification of the cell wall is common, generally show a loss in pigmentation and die. It is possible that the colorless cells would have survived and grown had an organic energy source been available. Two exceptions to the effect mentioned above were noted. They were cultures *Anabaena cylindrica*, in which the cells became bleached in the presence of inhibitory concentrations of bacitracin, and those of the diatom *Navicula pelliculosa*, in which inhibitory concentrations of nystatin resulted in lysis of the cells.

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BIOTIC RELATIONSHIPS BETWEEN SOIL ALGAE AND OTHER MICROORGANISMS[1]

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ABSTRACT

PARKER, BRUCE C., and HAROLD C. BOLD. (U. Texas, Austin.) Biotic relationships between soil algae and other microorganisms. Amer. Jour. Bot. 48(2): 185–197. Illus. 1961.—A study was conducted of biotic relationships between various algae and other microorganisms isolated from a sample of Texas soil. From 143 two-membered combinations of organisms tested in soil-water cultures, 3 were selected for detailed studies of the nature of the causal mechanisms of the associative effects. These were: (1) an association between a species of *Bracteacoccus* (Br. A–20) and a heterotrophic bacterium (B–6); (2) an association between a species of *Chlamydomonas* (Ch. 10) and a species of *Streptomyces* (Act–1); and (3) an association between a blue-green alga, *Phormidium* sp. (Ph. 14), and an as-yet-unidentified fungus (F–2). In the first association, the heterotrophic bacterium increased growth of *Bracteacoccus* up to 20 times in soil-water culture; the chief cause of stimulation was shown by a series of experiments to be the decomposition by the bacterium of complex nitrogenous substrates in the soil resulting in the release of simplified products which were available to the alga as a nitrogen source. In soil-water culture, *Streptomyces* (Act–1) enhanced the growth and motility of *Chlamydomonas* (Ch. 10) and induced akinetogenesis in the alga, while the actinomycete itself was stimulated in growth and production of conidia. The mutual stimulation was shown to be caused, in part, by carbon dioxide–oxygen interchange between the organisms. Motility of Ch. 10 was enhanced by a decrease in nitrogen as a result of the growth of Act–1 and by the ability of Act–1 to decompose and assimilate the extracellular polysaccharide of the alga. The assimilation of the extracellular polysaccharide by the actinomycete promoted its growth and conidia production. Initiation of akinetogenesis in *Chlamydomonas* occurred exclusively in close association with the filaments of *Streptomyces* in soil-water cultures, and only when the concentration of available nitrogen dropped below a critical level. The akinetogenic factor has not yielded to isolation and identification, but there is some suggestion that it may be an antibiotic substance. *Phormidium* (Ph. 14) was frequently antagonized and annihilated by fungus 2 in the soil-water medium. Attempts to extract growth inhibitors from filtrates of the fungal medium were unsuccessful. Indirect evidence suggested that perhaps the consumption of extracellular polysaccharide with concomitant release of organic acid by the fungus might be the factor inhibiting growth of *Phormidium*. Attempts to confirm experimentally the ecological significance for these biotic relationships are reported.

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THE LITERATURE is replete with studies of biotic influences among soil microorganisms, yet relatively few of these have included: (1) relationships between algae and other microorganisms isolated from the same habitat; (2) use of the original, natural medium for the experimental studies; and (3) detailed investigations of the biotic effects