

AN EXPERIMENTAL ASSOCIATION BETWEEN CHLORELLA XANTHELLA AND A STREPTOMYCES¹

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ABSTRACT

An association in the form of a lichen-like crust ("actinolichen") was obtained by seeding *Chlorella xanthella* cells and spores of a *Streptomyces* sp. isolated from soil on the surface of potato sections partially immersed in water at temperatures with a diurnal fluctuation of 9-19 C or 14-25 C, and with illumination continuous or during the hours of the higher temperatures. Sections of the crust showed a lichen-like organized tissue with layers of *Streptomyces* sp. spores, hyphae alone and associated hyphae and algae.

DES ABBAYES (1951, p. 136) states: "La limite des Lichens est une chose difficile à préciser et paraît être assez arbitraire" and later in the same paragraph: "Il existe en effet entre Algues et Champignons toute une gamme de relations plus ou moins apparentées à la symbiose lichénique."

Probably many organisms are potentially capable of entering into some sort of association that would be favorable to one or both of the partners but in natural environments conditions may not always be suitable for the development of such a relationship. Also the difficulties found in the experimental synthesis of lichens are very well known. Ahmadjian (1962) has reported his experimental investigations in the synthesis of *Acarospora fuscata*. Lazo (1961) found that the green alga *C. xanthella* was able to grow into full association with the plasmodia of two myxomycetes belonging to the family Physaraceae and form a living structure that Alexopoulos later named a "myxolichen" (1962). Parker and Bold (1961) studied the biotic relationships between soil algae and other microorganisms and obtained some interesting results. Lazo (1964) reported the formation of a lichen-like crust from an experimental association between *C. xanthella* and a *Streptomyces* sp. This present report is concerned with subsequent research on the production of this artificial lichen-like organ-

ism designated as an "actinolichen." This symbiotic system could serve as an experimental tool for those interested in studying the symbiotic relationships between different organisms and specially for those interested in the process of lichenization.

MATERIAL AND METHODS—*Streptomyces rimosus*, *S. griseus*, *S. coelicolor*, some unidentified streptomycetes, and one *Streptomyces* that belongs to the gray-spored series of species of the Section *Spira* were isolated from Chilean soils and cultured on a medium prepared with yeast extract, 6 g; malt extract, 3 g; K²HPO⁴ 1 g; agar 15 g; water to 1 liter. After 3 weeks when sporulation was satisfactory, one bacteriological loopful of spores from each one of these 14 streptomycetes, or occasionally small mycelial fragments, were then inoculated together with one bacteriological loopful of *C. xanthella* cells (N. 31 from the Indiana University Culture Collection of Algae, kindly supplied by Dr. R. Starr) that had been grown in malt extract-yeast extract agar of the same composition. The inocula were mixed carefully on the surface of different agar media (e. g. glucose-peptone, phosphate-peptone, malt extract-yeast extract, Czapek-Dox, potato and plain agar alone), potato broth and sand, potato broth and sawdust, and also on the surface of potato slices partially immersed in water. Tap water was used throughout. The potato broth was the supernatant obtained by boiling 200 g of potatoes in 700 ml of water. Potato broth was added to the sand or sawdust in enough quantity to soak completely these substrata, but an excess of broth was avoided on the surface of the sand or the sawdust. All agar media were about pH 6.8 before autoclaving. Potato sections were kept partially immersed in water in test tubes or petri dishes. Care was taken to avoid

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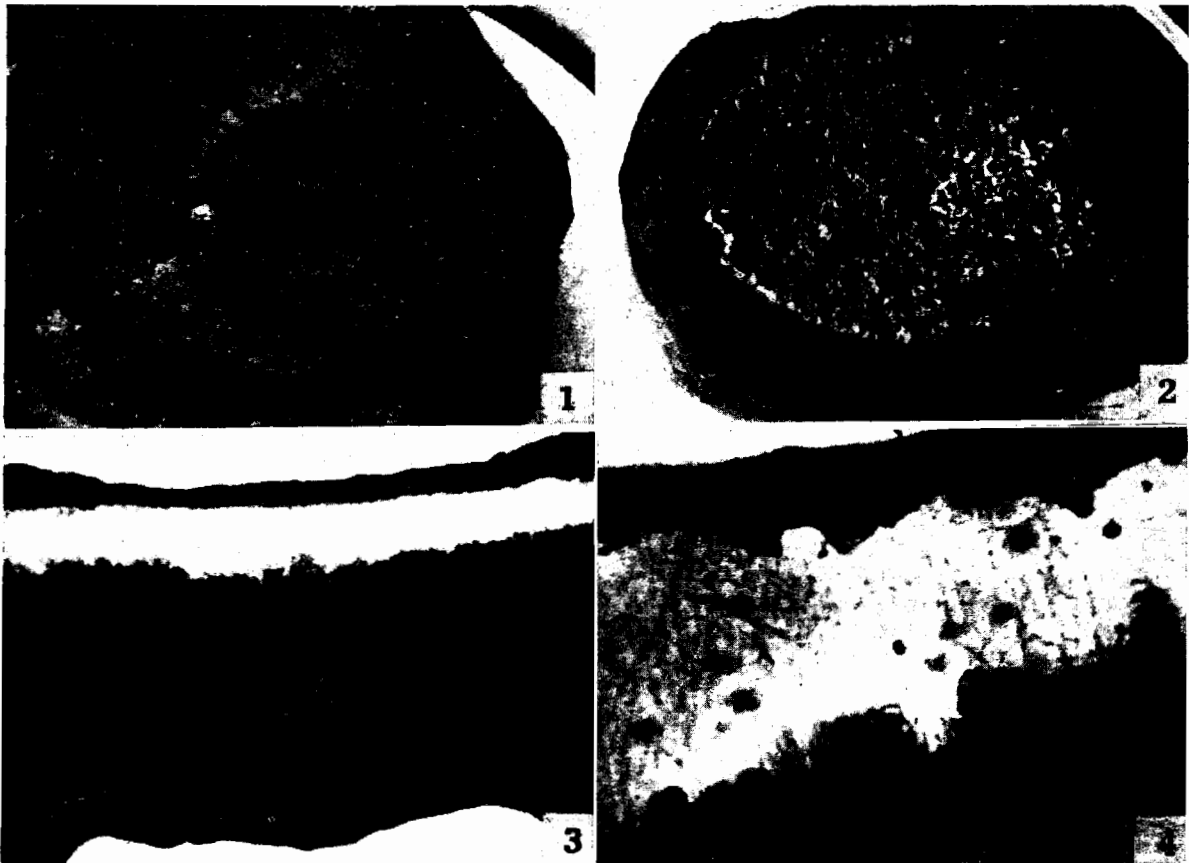


Fig. 1-4.—Fig. 1. *Streptomyces* NRRL B-3156 10 days old, grown on potato section partially immersed in water. Almost no sporulation except in one small zone, approx. natural size.—Fig. 2. *Streptomyces* NRRL B-3156-*Chlorella xanthella* “actinolichen.” Intense green color. Abundant sporulation, approx. natural size.—Fig. 3. Section of the “actinolichen” crust that shows one layer of *S. reptomyces* spores, one layer of hyphae alone, one green layer of *Chlorella* and associated hyphae and finally another area of spores, $\times 250$.—Fig. 4. Crust section that shows a layer of *Streptomyces* spores, one layer of hyphae and bunches of *C. xanthella*, $\times 750$

washing of the seeded surface of the potato section. The pH of the potatoes and the potato broth was about 6.5 before autoclaving. Some cultures were incubated at room temperature (9–19 C) and continuously illuminated by one Phillips fluorescent 20-watt lamp plus two 40-watt incandescent bulbs located 50 cm directly overhead. Other cultures were maintained in a greenhouse where the temperature ranged from 15–25 C with 16 hr of illumination per day (natural and artificial light). In the latter, the higher temperature occurred during the period of illumination.

RESULTS—Three kinds of interactions between the streptomycetes and *C. xanthella* were observed: (1) Some soil streptomycetes retarded the growth of the algae or destroyed them altogether; (2) In other cultures both organisms developed independently of one another; (3) One *Streptomyces* that belongs to the gray-spored series of species of the Section *Spira*² (Fig. 1) became associated

with the alga, the two growing together in a thallus or crust-like structure (Fig. 2) with each one located in a definite position. The stability of this association was demonstrated by the ability of small pieces of the thalloid formation to multiply when serially transferred onto fresh potato sections and to grow into new, large crusts with the same morphology and anatomy of the original parent inoculum.

The developmental behaviour of the alga and *Streptomyces* NRRL B-3156 is dependent to a large extent on the nature of the medium. In all agar media the two components invariably grew independently of one another. It was also observed that the antagonistic action of some streptomycetes against *C. xanthella* was more pronounced on nutrient agar. A lichen-like crust (“actinolichen”) with *C. xanthella* and *Streptomyces* NRRL B-3156 growing in a definite association with respect to one another formed only on the potato sections partially immersed in water and in the sand-potato broth, sawdust-potato broth media.

Sections of the “actinolichen” crust from 10

² This *Streptomyces* sp. was later sent to Dr. C. W. Hesseltine and codified as *Streptomyces* NRRL B-15 in the ARS Culture Collection of Peoria, Ill. 3

to 30 days old cut by hand with a razor blade showed that the dark-colored surface consisted of *Streptomyces* spores. Below this was a green layer made up of hyphae and algae resting on a mass of mycelium alone. Occasionally, however, part of the crust wrinkled and pushed up above the potato surface. When this happened spores were present on the sides as well as the top. Sections from such crusts (Fig. 3 and Fig. 4) showed a somewhat different morphology: there were a dark layer of spores, a green layer of associated hyphae and algae with bunches of enlarged *Chlorella* cells, a thin zone of hyphae alone and finally another area of spores. Studies on the histology and fine structure of the "actinolichen" are in progress. The type of contact between the *Streptomyces* hyphae and the *Chlorella* cells seems to be appressorium-like.

When an "actinolichen" was transferred to nutrient or potato agar, the crust disintegrated in that the *Streptomyces* and algae developed independently of one another. However, when fresh autoclaved potato sections partially immersed in water were used as a culture medium there was no disintegration and a new crust developed with the same characteristics of the original lichen-like organism.

At temperatures of 9–19 C the control cultures of *Streptomyces* NRRL B-3156 required about 20 days to form colonies that were visible with the naked eye. The crust formed by *Streptomyces* NRRL B-3156 and *C. xanthella* was visible in 10 to 11 days, however. Both cultures, that is the control and the crust, were kept under the same conditions. In the greenhouse at 14–25 C crusts developed in 3 days, growth again occurring about twice as rapidly as the alga-free controls that took 6 days to reach a comparable size. Temperatures above 26 C were adverse to crust formation because the *Streptomyces* NRRL B-3156 tended to overgrow the algae.

DISCUSSION—It has been possible to obtain a lichen-like ("actinolichen") crust formed by two organisms that have not been reported to associate in natural environments. It seems that the critical factor in producing the association is the use of an appropriate culture medium that restricts the spreading of the *Streptomyces* hyphae; effective media were potato sections partially immersed in water, sand or sawdust

soaked in potato broth. All of the tested agar media, even the one that contained the same potato broth used as a nutrient in the sand and sawdust media, were adverse to crust formation. Other important factors for obtaining the association are light, humidity and temperature which favor the growth of *C. xanthella* since the alga is the sensitive partner. It seems essential that optimum growth conditions be provided for it, while growth of the *Streptomyces* is restricted, if the "actinolichen" crust is to be established. Overgrowth by the *Streptomyces* hyphae occurred when light was not sufficient, at low humidity, when temperature rose over 26 C, or when both partners were seeded in any agar media where the *Streptomyces* NRRL B-3156 hyphae grew without restriction. The delicate equilibrium between the two components of the lichen-like crust is demonstrated by the facility with which they separate when explants from the crust are transferred to agar media.

Up to now it has not been possible to say that a true lichen (fungus-alga) can be obtained by the method used in the "actinolichen" formation, but if the mycobiont and the phycobiont of any lichen can have a balanced growth on the media and conditions in which the "actinolichen" was obtained it should be possible and perhaps easy to synthesize a real lichen. It also seems reasonable to predict that some fungus grown under these conditions would associate with *C. xanthella*, which has been demonstrated to be such a compatible mate with two myxomycetes and one *Streptomyces*, or with any other compatible alga.

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