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THE ACTINOMYCES OF THE SOIL.*

BY SELMAN A. WAKSMAN AND ROLAND E. CURTIS,

Research Assistants, Agricultural Experiment Station, Rutgers College.

The study of soil microorganisms has attracted the attention of many investigators, and great advance has been made in the study of bacteria, fungi, and protozoa. Very little attention, however, has been paid to the actinomycetes as a group of soil organisms. The present work has been undertaken with the purpose of demonstrating the occurrence of actinomycetes in different soil types, at different depths, and under different cultural and climatic conditions. An attempt has been made to secure a knowledge of the physiological activities of these organisms and their possible part in soil fertility.

During the past forty years about forty types of actinomycetes have been studied under different names and under varied environmental conditions. Most of these descriptions are so incomplete that it was found impossible to identify many of the organisms at hand with those previously studied. Almost all former investigations have been undertaken from a pathological standpoint, and the descriptions have been adapted to that purpose. With one exception, the studies heretofore made on soil actinomycetes have been limited to a very few representatives of this group.

The present paper records an attempt to study the occurrence of actinomycetes in the soil, and to classify them according to their morphological and physiological characters; it is necessarily incomplete, inasmuch as the sources of literature for study and identification are so limited and the field of investigation is so large.

HISTORICAL.

Cohn (5), in 1875, was the first to study an actinomycete, under the name of "Streptothrix" Foesteri. Bollinger (2) found one in an "actinomycose" swelling of cattle, and named it Actinomycetes for its radiating form. Rossi-Doria (19) studied *Streptothrix alba*, *Str. nigra* (*Str. Foes-*

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teri), *Str. albido-flava*, *Str. violacea*, *Str. carnea* and *Str. aurentiaca*. Gasperini (9) made an important contribution to the knowledge of actinomyces by his work on *Act. albus*, *Act. sulphureus*, *Act. luteo-roseus*, *Act. asteroides*, *Act. carneus* and *Act. aurentiaca*. More work followed: names were mixed and the terms "Actinomyces," "Streptothrix," "Cladothrix" and "Oöspora" were often interchanged, all of them meaning the same type. Petruschky (18) united all fine mycelial, unseptate fungi into the family "*Trichomycetes*," and divided this into four groups: (1) Actinomyces, those forming a radiating growth when parasitic in animal tissues, (2) Streptothrix, having an abundant true branching, wavy or curly growth, late fragmentation and formation of conidial chains, which serve as organs for multiplication, (3) Cladothrix, having false branching, quick fragmentation and therefore bacillary character of old cultures, (4) Leptothrix, never shows true branching, but stiff, little curved hyphae on which no organs of division can ever be detected.

Lehmann and Newmann (14) consider the actinomyces a special group, which stands between the Hyphomycetes and Schizomycetes; related to the latter by their slender hyphae and protoplasmic properties, and to the former by the branching formation of aerial hyphae with conidia-like structures. They are defined as "delicately threaded organisms, with true branching, in part very abundantly ramifying mycelium, partly with the formation of conidia. There is a tendency to the formation of clubs or knobs at the ends of threads." This family is divided into two groups. Group I contains the corynebacteria (L & N), which are "slender, often somewhat bent rods, usually having a tendency toward a clubbed swelling at the ends, branches rarely observed in young cultures, easily broken off; always non-motile, conidia never found"; and Mycobacteria (L & N), with "clubbed swellings rare in cultures, in tissues somewhat more common, staining with difficulty or not at all." Group II, the Actinomyces, are described as follows by Hartz: "Mycelial threads long, thin; extending or winding; dividing without partitions, with delicate sheaths and true branching; many species separate from the hyphae rows of short spores (conidia) which, whitish and mold-like, project upward above the solid nutrient substratum. Motility sometimes manifested. Almost all varieties emit a musty odor."

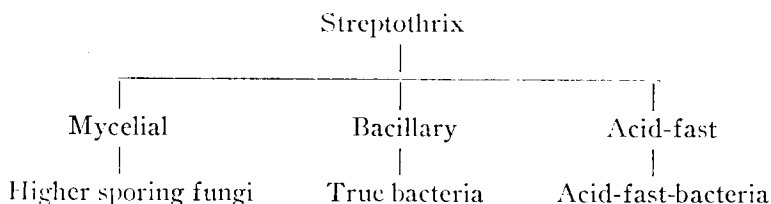
Sanfelice (21) very ably pointed out the faults in Petruschky's classification. He showed that actinomyces are, according to morphology and properties as revealed in culture, true and proper Streptothrices. The peculiarity, which seemed of so much importance to Petruschky that he separated the Actinomyces from the Streptothrix types, is only a specific property. The term "Actinomyces" as understood by Sanfelice, and by Lehmann and Newmann, will be used by the authors, since this term alone



can be applied to the great mass of the soil microorganisms which are discussed in this paper.

Musgrave (17) and others used the name *Streptothrix* for the genus. They define *Streptothrix* "as branching filamentous organisms, which develop into colonies made up of organisms and their transformation products. Terminal hyphae may or may not be radial, may or may not have clubs. This group is in general gram positive and many are acid fast." Foulerton (7) describes *Streptothrix* as "a tangled mass of branching mycelium. The mycelial stage is followed by segmentation and fragmentation, producing bacillary forms; and in artificial media by chain sporulation."

Claypole (4) gives the following tentative outline for the development of the microorganisms from the *Streptothrix* group:



She says, "The limits of species variation of the Streptothrices are neither set nor well known. The cause of confusion and for diverse opinions and practices lies in the extreme morphological and biological variability of these fungi. Some strains grow feebly on all media; some only on special media, and some apparently cannot be cultivated. Much variation is to be found in the morphology of any given organism. It changes its appearance with differing culture media. The well known granules or 'Drusen,' a branched mycelial mass, fragmentations into apparent bacilli and cocci, true spores as well as the minute structures left after chain sporulation, may be found in the life history of one species. It would seem biologically more reasonable to look upon this group of Streptothrices with their variable morphology and close relationships, as representing the ancestral type that gave both the higher fungi and the true bacteria and not as being themselves, higher bacteria."

Rullmann (20), the first to study the actinomyces in the soil, concludes that *Act. odorifer* causes the soil odor, which it also forms on media containing carbohydrates, but that it has no nitrifying ability.

Beijerinck (1) studied the existence of actinomyces in nature and their ability to form "quinone," an oxidizing agent. He found actinomyces in garden soil even at a depth of one meter, and in dune sand as deep as two meters; he found them also on the roots of many plants. He states that they inhabit the outside cells of the plants as saprophytes,

not as parasites. They are omniverous. They can live on media free from combined nitrogen, which they get from the air or distilled water (not atmospheric nitrogen), so small is their nitrogen requirement. They must play an important, if not a dominant, part in humus formation. He brought out the peculiar property of the actinomyces, namely, their power to reduce nitrates to nitrites without causing much loss of nitrogen. He also found that subsoils, though containing fewer numbers of actinomyces than the surface soil, are relatively richer in these than in other microorganisms. The only types studied by Beijerinck were *Act. albus* and *Act. chromogenus*.

Hiltner and Störmer (12) found that in the spring actinomyces form 20 per cent of the total bacterial numbers in the soil, while in the fall they form 30 per cent.

Fousek (8) found in the fall a greater percentage of actinomyces than in the spring. He found them to form 20 to 30 per cent of the organisms in loam soils, 8 to 15 per cent in clay soils, and 7 to 10 per cent in sands. Fallow soils contained larger numbers than cultivated soils. The actinomyces assimilate nitrates, ammonia and amido nitrogen, and form ammonia from organic substances. Nitrates are reduced to nitrites; free nitrogen is not assimilated. He finds *Act. albus* and *Act. chromogenus* to be predominant among the soil actinomyces.

Hagem (10) isolated four actinomyces from the soil, but from his short description of the macroscopic appearance of the organisms, one can hardly get a true idea as to which species he really had.

Münter (16) isolated seven organisms from different soils: *Act. odorifer*, *Act. chromogenus*, *Act. albus* I and II, and three more organisms which he terms *Act. S-a*, *Act. S-b*, and *Act. S-c*. He finds that these organisms can assimilate sugar and organic salts; and that organic substances have a strong influence on pigment production. The organisms are very sensitive to acids and alkalies. All of them liquefied gelatin, with or without the production of a brown pigment.

Conn (6) found that actinomyces may make up as much as 40 per cent of the soil bacteria.

The most complete work on the actinomyces of the soil is that of Krainsky (13), who has given a full description of eighteen well characterized and defined species of soil actinomyces. The organisms have been studied on different media and rather complete morphological and physiological qualities are recorded. All of them reduce nitrates to nitrites to a greater or less extent. Krainsky studied the production of enzymes, as did Münter and several others. Some of Krainsky's organisms are reported as strong cellulose destroyers. On allowing plates of calcium malate agar to incubate for thirty days he found 20,800 colonies of actino-

myces per gram of soil, which was 30 per cent of the total number of organisms developing on this medium. The upper soil layer was found to be poorer in fast growing forms than the soil at the depth of fifty centimeters. Krainsky concludes that the actinomyces play an important part in the decomposition and humification of plant remains in the soil.

EXPERIMENTAL.

I. METHODS OF STUDY.

1. *Soils used.*

Seven soils have been considered in this work. They represent different types, from several localities and under differing climatic and cultural conditions. The principal purpose was to determine whether there are any so-called soil actinomyces, whether organisms isolated from one locality are found in another, and whether there is a constancy in the occurrence of the particular species. Three soils from the eastern Los Angeles County, California, were among those used. The samples from these represent a composite of the surface eight inches. They are: (1) an upland, residual, loam unirrigated and cropped for grain and hay, which will be designated as "upland soil"; (2) a heavy adobe soil, irrigated, from an orange orchard, termed "adobe soil"; (3) a sandy loam, irrigated, also from an orange orchard, termed "California loam." A fourth soil was secured from the experimental farm of the Oregon Agricultural College at Corvallis, Oregon. This is an adobe type and has been cropped to legumes and small grains, termed "Oregon adobe." The three remaining types were taken from the experimental grounds of the New Jersey Agricultural Experiment Station at New Brunswick, N. J., (5) a Sassafras sandy loam, heavily manured every year, under garden crops, and termed "garden soil"; (6) a Sassafras sandy loam unmanured for the past twenty years, under orchard, termed "orchard soil"; and (7) a heavy clay soil, under permanent meadow, termed "meadow soil." Samples from the last three soils have been taken at depths of 1, 4, 8, 12, 20 and 30 inches. All sampling has been done under sterile conditions, and in the subsequent handling and plating the usual bacteriological precautions against air or other contaminations have been observed. The New Jersey soils were plated out within a few minutes after the samples were taken. In the case of the Oregon and California soils the necessary time for shipment of course elapsed between sampling and plating.

2. *Media used.*

Most investigators have used beef extract agar and gelatin for the study of actinomyces. As was pointed out before, the actinomyces will grow readily on any medium containing enough carbohydrates. Their nitrogen requirements are very small. Beef extract agar and gelatin are

not suitable media for the culture of actinomycetes, because, first, they are rich in nitrogen and for this reason do not bring out the characteristic colors of the organisms, aerial mycelium is not readily formed, and when it is produced, is of a chalky color; furthermore, most species tend to produce colonies more or less white in color accompanied by a brown pigment in the substratum. Second: These media are not constant in composition, and the growth and color of the actinomycetes, two of the most important factors in their differentiation, are very sensitive to change in the composition of the medium. The above probably accounts for the fact that early investigators reported one *Act. albus* and one *Act. chromogenus* in the soil.

Krainsky has used in his work a calcium malate agar and several other synthetic media. For this, as in any bacteriological work, media of constant chemical composition are desirable. As actinomycetes may change considerably in color production and character of growth from the mere process of transferring several times on the same medium, it can be readily seen that different media, as well as those of varying composition, would give very incomparable results.

Brown's (3) albumen agar, slightly modified, has been used for the isolation of the organisms from the soil. Suitable dilutions, varying from 1:200,000 for the surface soils to as low as 1:10,000 for the deeper subsoils, were used. The plates were allowed to incubate for 14 days at 22° C. Counts were then made and the actinomycetes transferred to Czapeck's solution agar. On this latter medium macroscopic and microscopic studies of all the organisms were made. Each organism was also studied on potato plugs (incubated at 30° C.) and on 15 per cent gelatin, in distilled water (incubated at 15° to 16° C.). Some species were studied on Czapeck's solution, where a characteristic growth is produced; and on 1 per cent dextrose broth, for gas production. A few were studied on beef extract agar, mannite agar, and several other media.

All the actinomycetes studied liquefy gelatin. They seem able to get all necessary food from the pure gelatin in distilled water, decomposing the gelatin in all probability by means of an enzyme. The various species show marked differences in the rapidity with which they liquefy gelatin. Some form a liquid ring of 1 to 2 cm. diameter in three days; others hardly form a liquefied circle of 2 mm. diameter in ten days. There is also a difference in color production on gelatin. In this connection the actinomycetes could be divided into two groups: those that do not produce any color on gelatin, the liquefied portion remaining pure white; and those that produce a pigment (usually brown) in and around the liquefied portion. The depth of the pigment varies somewhat in the different species. Some of the organisms produce aerial mycelium on the gelatin. This appears to be characteristic of the particular species.

3. Numbers of Actinomyces in the Soil, and Their Relation to Numbers of Bacteria.

One of the authors (23) has pointed out the fact that, though the numbers of actinomyces decrease with soil depth, their numbers, relative to those of bacteria and fungi, greatly increase. At the depth of one inch the actinomyces made up from 7.3 to 12.1 per cent of the total number of microorganisms; at the depth of 30 inches they constituted 52.7 to 83.6 per cent of the total numbers.

Additional data are presented in the following tables.

TABLE I.
BACTERIA AND ACTINOMYCES IN NEW JERSEY SOILS.

Soil Depth in inches	Garden Soil				Orchard Soil				Meadow Soil			
	Bacteria		Actinomyces		Bacteria		Actinomyces		Bacteria		Actinomyces	
	Number	%	Numbers	%	Numbers	%	Numbers	%	Numbers	%	Numbers	%
SEPTEMBER 15, 1915.												
1	7,870,000	93.7	533,000	6.3	7,000,000	92.9	533,000	7.1	8,600,000	90.9	867,000	9.1
4	6,400,000	87.3	933,000	12.7	6,200,000	88.6	800,000	11.4	7,200,000	85.0	1,267,000	15.0
8	3,670,000	93.2	267,000	6.8	2,930,000	92.6	233,000	7.4	3,933,000	86.8	600,000	13.2
12	1,867,000	91.0	140,000	7.0	1,353,000	90.6	140,000	9.4	767,000	81.6	173,000	18.4
20	320,000	62.1	193,000	37.6	140,000	52.6	126,000	47.4	320,000	63.2	187,000	36.8
30	113,000	31.3	247,000	68.6	53,000	29.6	126,000	70.4	153,000	50.0	153,000	50.0
NOVEMBER 2, 1915.												
1	1,700,000	87.0	700,000	13.0	1,600,000	88.5	600,000	11.5	15,400,000	92.2	1,300,000	7.8
4	4,500,000	85.0	800,000	15.0	4,500,000	77.6	1,300,000	22.4	7,000,000	86.4	1,100,000	13.6
8	3,500,000	74.5	1,200,000	25.5	1,560,000	76.5	480,000	23.5	1,710,000	79.9	430,000	20.1
12	720,000	62.1	430,000	37.9	670,000	59.0	470,000	41.0	1,040,000	79.4	270,000	20.6
20	210,000	42.0	290,000	58.0	130,000	19.4	540,000	80.6	690,000	67.0	340,000	33.0
30	160,000	24.0	510,000	76.0	90,000	16.4	460,000	83.6	160,000	44.6	200,000	55.6
NOVEMBER 30, 1915.												
1	5,300,000	85.5	900,000	14.5	4,800,000	86.6	700,000	13.4	8,100,000	93.7	550,000	6.3
4	4,300,000	84.3	800,000	15.7	3,200,000	82.0	700,000	18.0	4,500,000	86.5	700,000	13.5
8	3,600,000	75.0	1,200,000	25.0	1,800,000	81.8	400,000	18.2	1,700,000	70.8	700,000	29.2
12	725,000	82.0	160,000	18.0	680,000	74.7	230,000	25.3	750,000	85.2	130,000	14.8
20	160,000	39.0	250,000	61.0	330,000	58.9	230,000	41.1	30,000	33.3	60,000	66.7
30	90,000	35.6	170,000	64.4	250,000	51.0	240,000	49.0	50,000	50.0	5,000	50.0

GENERAL AVERAGE FOR ALL THREE SOILS.

Soil Depth	Bacteria		Actinomyces	
	Numbers	%	Numbers	%
1 inch	7,340,000	90.8	743,000	9.2
4 inches	5,300,000	85.0	933,000	15.0
8 inches	2,710,000	81.6	612,000	18.4
12 inches	950,000	79.9	239,000	20.1
20 inches	259,000	51.3	246,000	48.7
30 inches	124,000	34.6	240,000	65.6

The data presented in Table I bear out well the observations of previous investigators, in regard to the distribution of actinomyces in the soil and their numbers, relative to those of bacteria.

The numbers of both actinomyces and bacteria are greatest in the surface soil, but while the bacteria decrease rapidly below a depth of four inches, the numbers of actinomyces are practically constant at depths from 8 to 30 inches. This point is graphically brought out in Fig. 1, where the average percentages of bacteria and actinomyces from Table I are plotted.

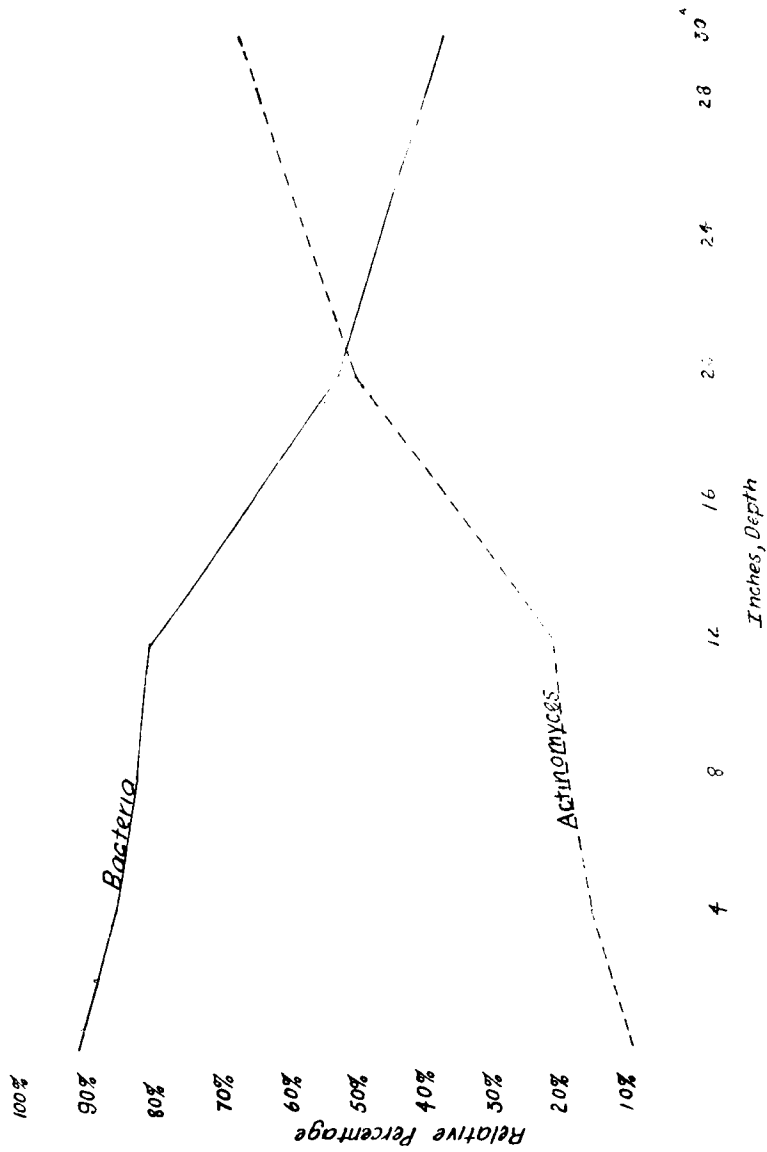


Figure 1—Relative percentages of Bacteria and Actinomyces at Different Soil Depths.

TABLE II.
BACTERIA AND ACTINOMYCES IN OREGON SOIL.

Depth	Bacteria		Actinomyces	
	Numbers	Per cent	Numbers	Per cent
0—2 inches	13,100,000	84.6	2,400,000	15.4
3—5 inches	10,600,000	63.1	6,200,000	36.9
10—14 inches	2,960,000	63.8	1,680,000	36.2
18—22 inches	680,000	48.6	720,000	51.4
28—32 inches	800,000	64.6	440,000	35.4

Table II presents the numbers and percentages of actinomyces and bacteria in the "Oregon adobe" soil. A close resemblance is seen between the percentages of bacteria and of actinomyces when the figures are compared with those in Table I. This is particularly true of the "meadow soil" and the "Oregon adobe." As the climatic conditions in the two places are very similar and the two soils have much in common, e. g., fine texture, large water capacity and a high water table, the similarity in results was to be expected.

TABLE III.
BACTERIA AND ACTINOMYCES IN CALIFORNIA SOILS.

Soil	Bacteria		Actinomyces	
	Numbers	Per cent	Numbers	Per cent
Upland (Sample I)	1,935,000	80.4	380,000	19.6
Upland (Sample V)	2,310,000	55.0	1,890,000	45.0
Upland (Sample VI)	2,420,000	62.7	1,445,000	37.3
Sandy Loam	6,010,000	80.8	1,430,000	19.2
Adobe	3,620,000	78.0	800,000	22.0

Table III gives similar data obtained from the southern California soils. As was noted before, these samples were a composite of the surface soil to a depth of 8 inches. A comparison of these figures obtained from semi-arid soils with those from humid soils brings out striking differences. In the surface eight inches of the New Jersey soils studied, actinomyces make up an average of 14.2 per cent of the micro-flora, exclusive of the higher fungi; while in the California soils this average is 28.6 per cent. This seems to indicate that semi-arid surface soils are relatively much richer in actinomyces than are those of humid regions.

MORPHOLOGY OF THE ACTINOMYCES.

The actinomyces grow on artificial media in the form of small, slowly developing, usually round colonies, which are partly submerged and partly aerial. Most of them usually begin to develop from the bottom of the plate, slowly pushing upward their rounded, glossy surface, until, on reaching the surface of the medium, they usually become covered with an aerial mycelium, which reminds one of lower spore-forming fungi. The aerial mycelium forms aerial spores which serve for reproduction. This

typical differentiation between the substratum and aerial growth, and the characteristic coloration of the colony and the aerial mycelium, which is of great importance as a basis for the separation of types, is had only under favorable conditions: in the presence of air and the proper medium. The colony is not smeary as in the case of most bacteria; the growth is solid and discrete. It can easily be lifted from the plate with a platinum needle, without breaking the colony. Placing the plate under the microscope, one can easily tell whether the organism is an actinomyces or bacterium, by the radiation of the thin hyphae from the colony into the medium. The surface of the growth is either smooth or undulated, ridged, folded, and even exfoliated, the colony having sometimes a lichenoid appearance. The edge is usually entire and filamentous. The substratum growth consists of very fine, branching mycelium, reminding one of bacterial structure, as there is no differentiation among wall, protoplasm, and cell sap. Septa are not formed and the younger branches are formed irregularly on the older ones. The aerial mycelium may cover only the central part of the colony, leaving a free margin, but very often the whole colony is covered with aerial mycelium. The latter usually consists of thicker hyphae than the substratum mycelium. They often end in club-like structures, or in spirals of different curvatures. Some of the species form spirals abundantly, the latter being generally characteristic of the type. The aerial hyphae break down into spherical, oval, or rod-shaped spores, of different sizes, and which are often united in pairs or in chains. Some organisms produce spores at an early stage, some only after they are several weeks old, some species do not form spores at all. The color of the aerial mycelium is characteristic of the species; sometimes it may be two-colored, at first white, only later developing the characteristic color. The color of the mycelium changes with the different media used, being influenced by the carbon and the nitrogen source. The colonies themselves are either colorless, or yellow, red, green, brown, black, blue, or of some other color. Many organisms produce a soluble pigment, which colors the medium, and this is also characteristic of the species. The three "violaceus" types produce a violet, blue or dark pigment on Czapeck's agar, while on potato only the "violaceus-ruber" produces the blue pigment, the other two species producing no pigment at all.

The growth on gelatin has been described before. Krainsky (13) found only the *Act. citreus* to produce an aerial mycelium on the bouillon gelatin; the present work has shown that many species produce an aerial mycelium on pure gelatin in distilled water, though not so readily as on Czapeck's agar.

Liquid media give characteristic growths with different species. All the organisms could be classified into four groups by their growth in

Czapeck's solution, as follows: those producing (1) a flaky growth on the bottom of the flask; (2) flaky growth all through the medium; (3) individual, well defined colonies all through the medium; (4) combined flaky growth on the bottom and colony formation through the medium. The character of colony formation may be specific. With some species the colonies are attached to the wall of the flask; others form colonies which always float free. Colonies may unite in masses on the surface of the liquid or at the bottom of the flask.

Dextrose broth (1 per cent) in fermentation tubes was inoculated with a considerable number of the species studied in order to determine their gas production. Not only was no gas produced in any case, but every organism failed to grow in the closed end of the tube. This fact shows that the actinomycetes are not preferential anaerobes. This has already been pointed out by Beijerinck, who classed them as facultative anaerobes.

An interesting point in the growth of some actinomycetes is the production of rings in the colony. This is especially prevalent on poor media. The ring formation may take place in the vegetative portion of the colony as well as in the aerial mycelium. So far no adequate explanation has been found for this phenomenon.

CLASSIFICATION OF THE ACTINOMYCES.

Most of the work on the identification of the actinomycetes has been undertaken from the pathological point of view. Different media have been used and different characters recorded. The result is, that with few exceptions, it is impossible to identify soil organisms with those that have been described before. For example: "*Act. chromogenus*" was supposed to be a well defined organism, yet Krainsky (13) had four representatives of the group. The authors have eight "chromogenus" types, each with such well defined characters as to make it almost impossible to classify them as one species.

The only work that could be satisfactorily used for the classification of the organisms at hand was that of Krainsky (13). Sanfelice (21) has classified the actinomycetes into three groups: (1) *albus*, (2) *flavus*, (3) *violaceus*. This grouping, based merely on the color of the colony, is purely arbitrary, because, as has been pointed out, the color varies with the media used. Krainsky classifies the actinomycetes into: (1) the Macro-group, which appear early on the plates, form large colonies, and bear oval or spherical conidia. (2) the Micro-group, appearing late, in small colonies, and producing spherical conidia. They are strong cellulose decomposers. (In this group belong the organisms producing violet and yellow colonies.)

This method of grouping is far from perfect. The size of the colony

and the time of its appearance on the plate depend not only on the organism itself, but on the media used and the incubation temperature. Also, the fact that both groups may produce spherical conidia is confusing.

In the present work it was thought advisable to describe the organisms as to their characteristic growth on different media. When sufficient material has accumulated it may be possible to work out a system of classification having for its foundation the more stable and important characters of the organisms.

While the grouping of the organisms according to the rapidity with which they liquefy gelatin and according to their production or non-production of pigment in this medium, does not meet all the objections to such a classification, it is believed by the authors that it offers a starting point for identification. The term "rapid liquefaction" is here applied to liquefaction of 15 per cent gelatin in distilled water, in three days at 15° to 17° C.

Over one hundred organisms have been isolated from the soil. These represent 30 species described in the present article. Several organisms have produced such scanty and uncharacteristic growth on the media used that it has been thought advisable to keep them under observation for a longer period, in the hope of detecting important stages in their life history.

DESCRIPTION OF THE ACTINOMYCES ISOLATED FROM THE SOIL.

Act. violaceus, n. sp.

Czapeck's Agar. Colony at first colorless, turning red and blue, colors seen very clearly in the reverse. The red color is soon absorbed by the profuse production of a cyanine blue (Rdg.* ix-51-m) pigment, which diffuses through the medium. Colonies 1 to 3 mm. in diameter, showing rapid growth and formation of zones. Surface of colony smooth with a narrow, entire, white margin. Aerial mycelium appears at an early stage of the colony, at first white, then turning to mouse-gray (Rdg. li-15'''). It has a silvery appearance due to the drops of water exuded upon the surface. Odor present, but weak. Microscopically two kinds of mycelium could be determined: the substratum mycelium consisting of fine closely branched filaments of a red to blue color; and the aerial growth consisting of thicker, straight filaments with very little branching at the edge of the colony, but more branching in the centre. Numerous spirals are found in the aerial mycelium. These as well as the hyphae break up readily into oval to rod-shaped conidia, 0.8 to 1.5 x 0.7 to 1.0 μ .

Gelatin. Spreading, dense, colorless growth, with an early production

* This abbreviation throughout this paper refers to Ridgway, "Color Standards and Nomenclature."

of a white aerial mycelium, underlaid by a pinkish coloration. Gelatin around the colony slowly liquefied, remaining clear.

Potato plug. After 36 to 48 hours, growth appears as a mass of well defined, round colonies, 1 mm. in diameter. White aerial mycelium is produced at an early date. Color of plug is at first unchanged, but after 4 to 5 days red and blue pigments are produced, either of them predominating; these also slightly color the white aerial mycelium.

Czapeck's solution. Growth consists of a flaky mass on the bottom of the flask, with numerous, small, round colonies all through the medium.

Glucose solution. A solid growth in the form of a grayish-white ring is formed on the surface, close to the side of the flask, with no growth through the medium. White to gray aerial mycelium appears at an early stage.

Hab. Isolated several times from the California adobe soil. Herbarium Nos. 8 and 44.

Act. violaceus-Caeseri, n. sp.

Czapeck's Agar. Growth very slow, consisting of gray colonies, 2 to 3 mm. in diameter. Surface of colony glossy and much folded. Aerial mycelium produced very late; it is pure white, with no shading into gray. A plum-purple (Rdg. xxiv-57-m) pigment is produced at an early stage, and gradually diffuses all through the medium. Pigment is much darker than that produced by *violaceus-ruber*, and no red tinge is ever observed. Medium becomes so dark from the diffused pigment as to be black by reflected light. Weak odor is given off. Microscopical examination shows the mycelium to consist of fine filaments, with the production of numerous open spirals in the aerial mycelium. Conidia could not be demonstrated.

Gelatin. Growth very small, with no aerial mycelium produced. Gelatin around the colony is rapidly liquefied, with no change in color.

Potato plug. Growth consisting of small yellowish colonies, which develop very slowly. Little aerial mycelium seen even after cultures are 20 days old. The color of the potato is not changed. No pigment is produced.

Czapeck's solution. Small flaky growth on the bottom of the flask. None through medium. All medium is colored blue at an early date. This is the only organism which colored deeply the Czapeck's solution.

Glucose solution. Thin, flaky growth on bottom of the flask, with none through medium.

Hab. Isolated once from the upland California soil. Herbarium No. 31.

Act. violaceus-niger, n. sp.

Czapeck's Agar. Colony at first dark gray, turning almost black. 2 to 4 mm. in diameter. Surface glossy, much folded with a very thin,

gray margin. A white to gray aerial mycelium is produced after the colony has well developed. A bluish black pigment is produced at a later stage of its growth. The pigment slowly dissolves in the medium, turning almost black. Odor fairly strong. Microscopically two types of mycelium were found: the thin, branching filaments of the substratum, and the thick filaments of the aerial mycelium. The aerial mycelium fragments not very rapidly, producing a few conidia, spherical and oval, 1.2 to 2.3 x 1.2 to 1.5 μ . These often occur in chains.

Gelatin. Gray growth on gelatin, with no production of aerial mycelium. Gelatin around colony rapidly liquefied, but without any change in color.

Potato plug. Growth at first very slight, but after 48 hours it develops in a yellowish-gray continuous thick smear, which turns brown at a later date. White aerial mycelium covers the growth late. Plug is not discolored.

Czapeck's solution. Colonies large, 2 to 3 mm. in diameter, appearing at the bottom and surface of the solution, but none throughout the medium. The colonies are bluish in color, with a regular margin. Medium is not colored.

Hab. Isolated once from the upland California soil. Herbarium No. 39.

Act. crithrochromogenus, Krainsky.

Czapeck's Agar. Colonies small, round, 2 to 3 mm. in diameter, with a slightly cut margin. Color of colony is at first tawny-olive (Rdg. xxix-17"-i) to a buffy-brown (Rdg. xl-17"-i). Surface smooth at first, then liehroid. White to gray aerial mycelium found at a later stage; this does not cover the whole surface, appearing only at separate parts of it. Reverse of colony dark. The production of soluble brown pigment at an early stage of its growth is characteristic of this organism as well as of all the other chromogenes species. The substratum mycelium consists of fine filaments with little branching. Aerial mycelium does not show any distinguishable structure. Conidia are abundant and fairly large, rod-shaped, 1.5 to 2.4 x 1.1 to 1.4 μ . A strong odor can be easily detected.

Gelatin. After several days of growth, each colony is found at the bottom of a small pit. Centre of colony is yellow, edge hyaline. The radial mycelium extends into the unliquefied portion of the gelatin. Slight white aerial mycelium is found on the surface of the older colonies. Gelatin around the colony is liquefied very slowly and is colored brown.

Potato plug. Growth consisting of small individual colonies, forming a continuous streak all over the plug. Colonies are at first yellow-gray, becoming with age dirty-gray and glossy in appearance. Aerial mycelium not formed readily. Plug is darkened.

Czapeck's solution. Growth consists of individual, round, brown colonies 1. to 1.5 mm. in diameter all through liquid and on surface.

Glucose solution. Very little growth takes place.

Hab. Isolated from the upland and adobe California soils. Herbarium Nos. 17 and 18.

Act. diastato-chromogenus, Krainsky.

Czapeck's Agar. The description of this organism coincides very closely with that given by Krainsky for the organism under the same name. Colonies fairly large (3 to 5 mm.), at first colorless, then becoming brownish. Gray aerial mycelium colors all the colony at an early stage without leaving any free margin. Small drops of water are exuded upon the surface. Reverse of colony is brown, and a light brown pigment is produced dissolving through the medium. Odor present, but weak. Microscopically, the aerial mycelium is found to consist of long filaments, with very little branching. These break up readily into rod-shaped conidia, 1.5 to 1.8 x 1. to 1.2 μ .

Gelatin. Gelatin is rapidly liquefied with the production of a brown color.

Potato plug. Growth light gray turning brownish, in one continuous streak, with several small separate colonies (1 mm.). White aerial mycelium appears at an early date and covers the whole growth. Potato is blackened.

Czapeck's solution. Flaky growth on the bottom of the liquid, and a pellicle on the surface. No growth through the medium.

Glucose solution. Flaky growth on the bottom of the liquid, and a ring of individual colonies 1 to 2 mm. in diameter on the surface of the liquid close to the glass of the flask.

Hab. Isolated twice from the California adobe soil. Herbarium Nos. 41 and 47.

Act. purpco-chromogenus, n. sp.

Czapeck's Agar. Colonies small, 0.5 to 1.5 mm. in diameter, developing very slowly. They are brown in color with a brown to black reverse. Surface of colony is glossy, and raised in the centre. A brown soluble pigment is produced, which shows a distinct purple tinge, when viewed by transmitted light. Aerial mycelium is formed later. When culture is over 4 weeks old a brownish purple to black surface mycelium is formed. Margin of colony is waxy-yellow in color and lichnoid in appearance. No odor could be detected. Microscopically, no difference could be seen between the substratum and surface mycelium, a condition which suggested the question whether or not the latter could be called aerial mycelium. No conidia could be found. Substratum mycelium seems to break up into spherical non-staining bodies, .75 to 1. μ in diameter (oidia?).

Gelatin. Growth very slow. Liquefaction of the gelatin is slow with the production of a brownish pigment only at a late period.

Potato plug. No growth on potato in 2 to 3 days at 30° C. Only after 10 days the colonies were found to be very small, orange colored, grouping in a bead-like fashion. Colonies become dark brown with age. Potato slightly colored brown.

Czapeck's solution. Flaky growth on the bottom of the flask.

Hab. Isolated once from the California adobe soil. Herbarium No. 49.

Act. viridochromogenus (?), Krainsky.

Czapeck's Agar. Colony 2 to 8 mm. in diameter, at first yellowish-gray, slightly raised above the substratum. Surface glossy, granular, at first yellowish-brown and finally becoming dark green in color. White aerial mycelium appears first at the edge of the colony, rapidly advancing toward the centre, until finally the whole colony is covered with a white-gray aerial mycelium. Margin of colony is colorless, regular. Reverse of colony is colored at first yellowish-gray, later becoming dark brown. A dark brown to black pigment is produced which dissolves through the medium. Microscopically, the aerial mycelium is found to consist of a dense mass of filaments with little branching. The filaments fragment easily into long pieces, and these usually break up into oval-shaped spores, 1.2 to 2 x 0.75 to 1.1 μ . Odor present but not very pronounced.

Gelatin. Gelatin rapidly liquefied with the production of a brown pigment.

Potato plug. Growth much folded, continuous streak, at first grayish yellow to green, but finally becoming dark green. White aerial mycelium is produced. Potato becomes black.

This organism seems to coincide with Krainsky's "viridochromogenus," though it differs from it in some details.

Hab. Isolated once from garden soil at a depth of 8 inches. Herbarium No. 61.

Act. chromogenus group.

Besides the four species of "chromogenus" previously described, several more organisms have been isolated, which show the characteristic features of the "chromogenus." These cannot be classified as one species, because they show distinct characters from one another. All of them are characterized by a colorless colony, changing later to different shades of brown. Surface mycelium is either absent or produced when culture is over 2 to 3 weeks old, and is of a brown to black color, hardly differentiated from the surface of the colony. Some organisms may produce small white tufts at a late stage. Reverse of colony is brown to black.

A brownish pigment diffuses through the substratum. The colonies are hard, a condition due probably to the production of quinone, which is characteristic of all the "*chromogenus*" species, as was pointed out by Beijerinck. Gelatin is liquefied at first, but the liquefaction does not advance far; the hardening up is also probably due to the quinone production. A brown pigment diffuses through the unliquefied portion of the gelatin. These cultures have shown the same general characteristics as the *Act. chromogenus* isolated from potato scab, a culture of which was borrowed from the plant pathology department of the New Jersey Agricultural Experiment Station.

STRAIN No. 1. Colonies 3 to 5 mm. in diameter. Surface smooth, dry, with lichnoid margin. Color yellow becoming overlaid by a dry, thin, black sheet, with the production of white aerial mycelium in centre of colony at a late stage. Tendency to grow in individual colonies, and not to form a solid streak. General outline of colony is yellow to black. Reverse dark brown to almost black, medium colored brown. Microscopically, aerial mycelium was found to be either lacking or very scarce. Surface mycelium consists of thick hyphae, but no clubs observed. Conidia few, oval to rod-shaped, 1.2 to 1.8 x 1. μ .

Hab. California upland soil. Herbarium Nos. 40 and 48.

STRAIN No. 2. Colonies 3 to 5 mm. in diameter, colorless at first, becoming later gray. Surface glossy, ridged, with folds radiating from the centre of the colony. White aerial mycelium appearing in small tufts at a late stage. This is easily observed on mannite lacking in nitrogen, and used for the study of nitrogen-fixing bacteria. Microscopically, aerial mycelium was found to be scant, with numerous dark staining granules in the hyphae. Small oval conidia fairly numerous, 1. x 0.6 μ . Filaments show a club-shaped appearance at the end.

Hab. California upland soil. Herbarium Nos. 45, 53 and 54.

STRAIN No. 3. Colony is of a dirty gray color, with an intensive ring formation. Scant white aerial mycelium develops when culture is 6 to 8 weeks old. Microscopically, aerial mycelium can hardly be differentiated; it shows a granular structure, with the formation of clubs at the end of the filaments. Some filaments are greatly enlarged. No conidia were observed.

Hab. Isolated once from garden soil 20 inches deep. Herbarium No. 36.

STRAIN No. 4. Colonies large, 5 to 8 mm. in diameter, at first gray, then becoming brown in color. Surface glossy, at first smooth, later becoming slightly wrinkled. Snow-white aerial mycelium is produced. No conidia and no clubs could be observed under the microscope. Aerial mycelium was found to be very dense.

Hab. California loam. Herbarium No. 22.

Act. exfoliatus, n. sp.

Czapeck's Agar. Colonies round, 2 to 3 mm. in diameter, of a Dresden-brown color (Rdg. xv-17'-k), with a wide sterile margin. Colony has a tendency to crack, and surface growth to exfoliate and peel off. The margin of the streak culture is peeled off, leaving medium free. Many cracks are found in centre of growth. White aerial mycelium is produced at an early date. A blue pigment is produced in the colony, not soluble in the substratum, but seen clearly through the aerial mycelium. Reverse of colony is brown to black. Microscopically, the aerial mycelium is found to be thick, 1.5μ in diameter. Conidia oval, 1.2 to 1.8×1 to 1.5μ . Odor weak.

Gelatin. Colony develops in the bottom of a liquefied pit, showing a dense yellow mycelium in the centre; edge of colony is hyaline. Radial mycelium extends into the unliquefied portion of the gelatin. White aerial mycelium is produced. Gelatin around the colony is slowly liquefied, with no color produced.

Potato plug. Growth continuous, thick, somewhat folded, at first colorless to gray, later becoming yellow. No aerial mycelium produced. Potato not affected.

Czapeck's solution. Many minute individual colonies all through medium and heavy pellicle on surface of liquid.

Hab. Isolated several times from the adobe and upland soils. Herbarium Nos. 20, 50 and 51.

Act. diastaticus (?), Krainsky.

Czapeck's Agar. Colonies 2 to 4 mm. in diameter, gray, later becoming colored pale yellow. Many rings are formed by growth of colony. Aerial mycelium drab-gray (Rdg. xlv-17""d), with small white tufts protruding in several places. Reverse of colony brown to black, with the deep brown mycelium penetrating deep into the substratum. This organism seems to answer Krainsky's description of the "diastaticus." However, no biochemical studies have been undertaken as yet to prove the identity of this organism. Microscopically, two kinds of aerial mycelium were found. The white mycelium is very dense, made up of straight, branching filaments. The brown aerial mycelium is made up of dense clusters of fine and narrow spirals. Oval conidia, 1.1 to 1.5×1 to 1.2μ . Odor weak.

Gelatin. Small colonies with white aerial mycelium produced at a late stage. Gelatin around the colony is rapidly liquefied, with no coloration.

Potato plug. Colonies small, 1 to 1.5 mm. in diameter, covering all the plug. Color of colonies white-gray with white aerial mycelium, which later becomes ash-gray. Each colony is pitted and raised 1 to 2 mm. above surface of plug. Potato darkened in two days.

Czapeck's solution. Flaky, colorless growth all through medium and on surface.

Glucose solution. Heavy gray ring of colonies on surface of liquid.

Hab. Isolated once from California sandy loam. Herbarium No. 13.

Act. albus, Krainsky.

This is a very common soil organism, isolated by Krainsky and reported by many investigators, as one of the few actinomyces found in the soil. The identification of this organism was based on Krainsky's description of the *Act. albus*.

Czapeck's Agar. Colonies 3 to 5 mm. in diameter, uniform in size, pale neutral-gray color (Rdg. liii-n. g.-d). White aerial mycelium produced early. Uniform growth on slant. Odor weak. Profuse spore formation. Conidia are both spherical and oval, 1.2 to 1.6 \times 1.1 to 1.4 μ .

Gelatin. White aerial mycelium develops readily on the surface of the colony. Gelatin is liquefied rapidly with the production of a brown coloration.

Potato plug. Thin gray to brownish streak covered with white aerial mycelium, which becomes gray with age. Potato is not colored.

Czapeck's solution. Growth consists of small, white, individual colonies, 1 to 2 mm. in diameter, on glass of flask. None through medium and on bottom.

Glucose solution. Small growth consisting of a white ring on surface close to glass of flask.

Hab. Isolated several times from the adobe soil and garden soil at a depth of 8 inches. Herbarium Nos. 6, 30 and 63.

Act. alboatrus, n. sp.

Czapeck's Agar. Colonies colorless and fairly large, 5 to 8 mm. in diameter. Surface of colony at first smooth, later becoming ridged, with folds radiating from the centre. White aerial mycelium covers the whole colony at an early date, leaving a narrow glossy margin, which has a lichnoid appearance. Small drops of water are exuded upon the surface. Reverse of colony is at first light brown, becoming with age dark reddish-brown. Strong odor is present. Microscopically, aerial mycelium consists of dense, closely branched filaments. These break up into long fragments, which have nothing in common with what is ordinarily understood as conidia. No true conidia observed.

Gelatin. Gelatin is rapidly liquefied, with no coloration.

Potato plug. Growth forms a thick, continuous streak, much folded, glossy, and of a white color. Potato does not change in color. A rose-colored aerial mycelium appears only after 20 days at 30° C.

Czapeck's solution. Flaky growth on the bottom of the liquid, with few small colonies on the surface.

Hab. Isolated once from the adobe soil. Herbarium No. 52.

Act. reticuli, n. sp.

Czapeck's Agar. Colony colorless, 3 to 5 mm. in diameter, becoming covered soon with a thin white cottony growth. This white aerial mycelium is characteristic of the organism. It is very fine, forming a woven net over the whole surface of the colony, with holes of about 0.5 mm. Reverse of colony creamy, later becoming brownish. Aerial mycelium abundant, consisting of filaments having no branches or very short ones. Spherical conidia are abundant, 1. to 1.4 μ . Odor weak.

Gelatin. Rapid liquefaction takes place with the production of a soluble brown pigment only after 6 days.

Potato plug. Growth consists of brown, numerous colonies, 0.25 to 3 mm. in diameter, all over plug. Colonies are pitted, and covered with white aerial mycelium. Potato darkened.

Hab. Isolated from upland and adobe soils. Herbarium Nos. 43 and 95.

Act. citreus, Krainsky.

Czapeck's Agar. Colonies 3 to 5 mm. in diameter, with centre raised above substratum. Color of colony varying from olive-yellow (Rdg. xxx-23'') to citron-yellow (Rdg. xvi-23-b). Aerial mycelium of same color as colony, covering all colony without leaving any free margin. Reverse of colony deep colonial buff (Rdg. xxx-20''-b) to a deep yellow color, becoming with age yellow brown. Color of medium is unchanged. Odor weak. Microscopically, the substratum mycelium is found to consist of fine, branching filaments. Aerial mycelium consists of short, much branching, and tangled filaments. Conidia numerous, spherical to slightly oval, 1.2 to 1.8 x 1.2 to 1.5 μ . They do not stain evenly, showing a clear centre.

Gelatin. Colonies very dense, yellowish, with white aerial mycelium. Gelatin around the colony is rapidly liquefied with no discoloration.

Potato plug. Growth appears as a gray to yellow thin smear, with white aerial mycelium. Color of potato unchanged.

Czapeck's solution. Slight flaky growth on the bottom of the flask, medium remaining clear.

Hab. Isolated several times from the orchard soil at depths of 1 inch and of 20 inches, and from the garden soil at a depth of 20 inches. Herbarium Nos. 29, 82 and 87.

Act. flavus, Krainsky.

Czapeck's Agar. Colonies 3 to 5 mm. in diameter, olive-yellow (Rdg. xxx-23'') in color, showing a characteristic ring formation. Growth on streak has a tendency to form individual colonies. Aerial mycelium light drab (Rdg. xvi-17'''-C), with a wide sterile margin. Reverse of colony yellow to olive-yellow; no soluble pigment produced. The microscope

shows tangled aerial mycelium with little branching. The mycelium is swollen at intervals, forming the characteristic club shaped filaments up to 3μ in diameter. Spores oval and spherical, 0.9 to 2×0.9 to 1.1μ .

Gelatin. Growth dense, light yellow in color, with a shiny surface; no aerial mycelium is produced. Liquefaction of the gelatin around the colony starts early, but does not advance very rapidly. A light brown pigment is produced.

Potato plug. A finely wrinkled continuous growth gray to brown in color is produced in two days. White aerial mycelium develops only after 8 to 10 days' incubation at 30° C. Potato colored brown.

Czapeck's solution and Glucose solution. Flaky growth on bottom of the flask, none through medium.

Hab. Isolated from upland and adobe soils. Herbarium Nos. 23 and 38.

Act. parvus, Krainsky.

Czapeck's Agar. Colony 1 to 3 mm. in diameter, of a honey-yellow color (Rdg. xxx-19"). Surface of colony smooth and glossy, long remaining without any aerial mycelium. Scanty gray-yellow aerial mycelium appearing only late. Reverse of colony brownish. Substratum mycelium fine, very dense, and tangled. Aerial mycelium consisting of thicker branching filaments. Conidia oval, 1.2 to 1.8×0.9 to 1.3μ . No odor could be detected.

Gelatin. Colonies yellow, slowly developing on the gelatin. Liquefaction of the gelatin around the colony advances slowly, with no pigment production.

Potato plug. Growth continuous, folded, with a lichnoid margin, gray to brown in color. White aerial mycelium produced at an early stage. Potato colored black.

Hab. Isolated from garden soil at a depth of 12 inches, and Oregon soil. Herbarium No. 76.

Act. griseus, Krainsky.

Czapeck's Agar. Colony round, 3 to 6 mm. in diameter, growing rapidly with the formation of numerous rings. Color of colony olive-buff (Rdg. xl-21"-d). Aerial mycelium appears at an early stage; it is a thick powdery mass of a water-green color (Rdg. xli-25"-d). The color of the aerial mycelium is somewhat lighter than that described by Krainsky. Odor present, but weak. Microscopically, the aerial mycelium is found to consist of long filaments, with very little branching. These fragment readily into rod-shaped conidia, $1.$ to $1.5 \times 0.8\mu$. The conidia occur often in chains; they do not stain readily in the centre; so that they produce a beaded effect.

Gelatin. Greenish-yellow colonies with a prominent substratum

growth. Aerial mycelium produced of a white-gray color. Liquefaction of the gelatin takes place rapidly with no pigment production.

Potato plug. Growth yellow, continuous, with the formation of individual colonies separated from the streak. Aerial mycelium at first white-gray, then changing into the characteristic water-green color. Potato is colored brown.

Czapeck's solution. Flaky growth on the bottom and throughout the medium.

Glucose solution. Heavy growth consisting of round colonies (1 to 3 mm. in diameter) floating on the surface and forming a ring in contact with the glass. A powdery white aerial mycelium soon covers the surface of the colonies. Some growth is found on the bottom of the liquid.

Hab. Isolated from California adobe soil. Herbarium Nos. 33 and 34.

Act. albo-flavus, n. sp.

Czapeck's Agar. Colonies small (2 to 4 mm.), round, colorless and glossy, at first, later becoming yellowish in color. Aerial mycelium forming a white powdery mass, with a yellow tinge developing later. Reverse of colony is yellowish, but medium is not colored. Under the microscope the aerial mycelium was found to have a tendency to produce special structures, consisting of a mass of hyphae massed together into a rope, and from this rope fine filaments coming out in the shape of side branches. The structure looks like the root of a tree and fine rootlets coming out on the side. This special structure persisted even in stained preparations. No conidia were observed. No spirals produced. Weak odor present.

Gelatin. Rapid liquefaction of the gelatin with no pigment production. The colonies are floating in the liquefied portion. No aerial mycelium produced.

Potato plug. Gray, continuous, thin growth. White aerial mycelium produced in 2 days at 30° C. Color of potato not changed.

Czapeck's solution. Few small colonies on the glass, none through medium or on bottom.

Glucose solution. White cylindrical colonies 3 x 1 mm., very uniform in size, growing together in a mass on the surface of the liquid. White aerial mycelium is produced at an early stage. The type of colony is very characteristic of this species, because it has not been observed in any other culture.

Hab. Isolated once from orchard soil at a depth of 20 inches. Herbarium No. 10.

Act. Verne, n. sp.

Czapeck's Agar. Colony 3 to 6 mm. in diameter, much folded, of an Isabella color (Rdg. xxx-19-i), with a wet surface. Margin of colony lichenoid. Aerial mycelium is little differentiated from the surface of the colony, and no such could be demonstrated. An elm-green (Rdg. xvii-27-

km) soluble pigment is produced, which diffuses rapidly all through the medium. Weak odor present. Microscopically, substratum mycelium found to be very fine, radial. Surface mycelium thicker and much branched. No segmentation or conidia could be demonstrated. Raised portion of old growth consists of a hard yellowish amorphous crust breaking into irregular fragments; there is no true aerial mycelium.

Gelatin. Globular colonies grow below the surface of the liquefied portion of the gelatin, varying in size from microscopic to 1.5 mm. No aerial mycelium is produced. Reverse of colonies is dark, due to the green pigment discoloring the medium. Gelatin is rapidly liquefied with no brown coloration.

Potato plug. Growth at first yellowish-gray, with a tendency to form individual colonies. Later it becomes thick and much folded. Scant white aerial mycelium is produced only on the tip of the growth. Color of potato is unchanged.

Czapeck's solution. Small colonies formed on the bottom of the liquid.

Glucose solution. Slight flaky growth on the bottom.

Hab. Isolated once from the upland soil. Herbarium No. 42.

Act. albosporeus, Krainsky.

Czapeck's Agar. Colony Acajou red (Rdg. xiii-l'-1), with an early production of white aerial mycelium. Reverse of colony orange-red. Growth on streak continuous, without any tendency to form individual colonies. Answers closely Krainsky's description. Substratum mycelium fine, radial, red colored. Aerial mycelium coarser, white, much branched. No spirals produced. Conidia very distinct, formed readily; spherical and oval shaped 1. to 1.8 x 0.8 to 1.2 μ , often occurring in chains.

Gelatin. Colonies at first yellow in centre and hyaline at the margin; later they become red colored, remaining hyaline at the margin. Gray mycelium is produced in the centre of the colony. Gelatin is rapidly liquefied, with no pigment production.

Potato plug. Growth very slight, translucent, gray, becoming orange colored, with white aerial mycelium.

Czapeck's solution. Small flakes all through the medium and on the surface with the production of a soluble rose pigment.

Glucose solution. A pinkish ring is formed at the surface in contact with the glass; fair growth downward in the medium.

Hab. Isolated once from the upland soil. Herbarium No. 26.

Act. Bobili, n. sp.

Czapeck's Agar. Colonies small, 1.5 to 2.5 mm., folded, with a much cut lichnoid margin. Color at first coral red (Rdg. xiii-5'), becoming with age Acajou to Pompeian red (Rdg. xiii-l'-3'i), with a light colored margin. Reverse is light red. No true aerial mycelium is produced.

Odor strong, mouldy. Microscopically, the surface mycelium was found to be very fine and dense. No true aerial mycelium or spores could be demonstrated. Old surface growth consisting of a mass of degenerated mycelium.

Gelatin. Colonies dense, some having slight aerial growth. Each colony found at the bottom of a liquefied pit. Gelatin rapidly liquefied, at first colorless, then becoming colored brown. Under the microscope peculiar hyaline aerial formations were observed in the form of wedge, up to 10μ wide at the base, and tapering to a point. When these were smashed under the cover slip, nothing more could be found.

Potato plug. Growth gray to red, spreading all over plug. Surface of growth dry and much folded. Scant white aerial mycelium formed late at the tip of plug. Potato becoming brown with age.

Czapeck's solution. Colonies, 2 to 3 mm. in diameter, found all through liquid and on the bottom. Colonies are colorless at first, later the centre of the colonies becomes orange.

Glucose solution. Flaky growth on bottom, none on surface.

Hab. Isolated from adobe and garden soils. Herbarium Nos. 15 and 37.

Act. Californicus, n. sp.

Czapeck's Agar. Colonies small, 1 to 2 mm., round, vinaceous colored (Rdg. xxvii-1"-d), growing deep into the substratum with almost no surface growth. The growth of the mycelium into the substratum can easily be followed by the red growth penetrating, 1 to 2 cm. deep, into the medium. No soluble pigment is produced, the color is found in the medium only where the mycelium has penetrated. Light neutral gray (Rdg. liii-n. g.-C) aerial mycelium covers surface in the form of a dry, powdery, thin layer. Microscopically, an abundant formation of open spirals could be found, 3.5 to 6μ in diameter. These break up easily into perfectly spherical conidia, which are very uniform and quite numerous, 1.2 to 1.5μ in diameter, often occurring in long chains. No odor could be detected.

Gelatin. Dense colorless colonies are abundant, with a gray-white aerial mycelium. Gelatin around the colony is slowly liquefied, with no pigment production.

Potato plug. Growth at first yellow to red, glossy, and shiny, with age becoming reddish-brown. No aerial mycelium produced. Potato not changed in color.

Czapeck's solution. Thin flaky growth all through medium and on surface.

Glucose solution. Pinkish ring formed at the surface, close to the glass. Scant flaky growth through medium.

Hab. Isolated once from the California sandy loam. Herbarium No. 3.

Act. Lipmanii, n. sp.

Czapeck's Agar. Colony 3 to 5 mm. in diameter, at first colorless, later becoming light brown. Centre of colony is elevated, giving it a conical appearance. There is an abundant production of aerial mycelium, which changes from neutral-gray (Rdg. liii. n. g.) to gray (Rdg. liii-6), with a white margin and white tufts all over surface. A ring formation takes place, alternating gray and white zones. Surface is smooth or slightly ridged. Minute drops of water exude upon the surface, giving the growth a silvery appearance. Reverse of colony is first light brown, then changes to almost black. No soluble pigment is produced. Under the microscope the aerial mycelium is found to be much branching and fragmenting readily into spherical or oval conidia. The conidia are 1. to 1.5×0.8 to 1.1μ , often occurring in chains. Odor weak.

Gelatin. Growth colorless, with white-gray aerial mycelium. Gelatin is rapidly liquefied with no pigment production. One strain of this organism (4) produced a cerro-green (Rdg. v-27-m) growth with white aerial mycelium.

Potato plug. Growth on the potato varies slightly with the different strains of the organism. It is usually a folded white to brownish smear, turning gray to gray-green. Aerial mycelium is white to ashy-gray. Color of the potato remains unchanged. The smear of one strain (12) is sulphur-yellow, of another (4) olive-green; otherwise the characters are alike.

Czapeck's solution. Flaky growth on bottom and surface, small white colonies all through the liquid.

Glucose solution. Grayish-white ring formed at the surface, close to glass.

Hab. This very common soil organism has been isolated by the writers many times from different soils, from the adobe, California sandy loam, and garden soil at a depth of 30 inches. Herbarium Nos. 4, 5, 7, 12, 58 and 62.

Act. Rutgersensis, n. sp.

Czapeck's Agar. Colony 3 to 8 mm. in diameter, slightly raised in the centre. Substratum mycelium penetrates deep into the medium. Surface colorless with an irregular margin. Aerial mycelium is produced at an early stage; it is at first gray, later becoming pale gull-gray (Rdg. liii-c. g). Zonation takes place in the formation of alternate gray and white rings. Reverse of colony changes from white to brownish. Microscopically, the aerial mycelium is found to consist of long, branching filaments, loose at the margin, but dense in the centre. There is an abundant spiral production of the close and open type. Conidia spherical and oval, 1. to 1.2μ in diameter, having a tendency to bi-polar staining. A strong odor is produced.

Gelatin. Rapid liquefaction of the gelatin takes place with no pigment production. No aerial mycelium formed.

Potato plug. Growth varies from a gray to a dark solid streak with scant formation of white aerial mycelium. Color of potato not changed.

Hab. This form, another common soil organism, has been isolated repeatedly by the writers from the local soils, from garden soil at depths of 1, 20 and 30 inches, orchard soil at 4 and 20 inches, timothy soil at 4 inches. Herbarium Nos. 67, 75, 79, 80, 83, 86 and 91.

Act. aureus, n. sp.

Czapeck's Agar. Colony 4 to 5 mm. in diameter, and translucent in color when 7 days old. Surface smooth. Aerial mycelium appears at an early date, at first mouse-gray (Rdg. li-15'''), then changing into a cinnamon-drab color (Rdg. xlv-13'''). Ring formation takes place by the alternation of white and drab colored zones. Reverse at first white, changing to brown and almost dark brown. No soluble pigment produced. A characteristic exudation of dirty gray drops of water takes place in the centre of the colony, forming a small ring. Weak odor present. Aerial mycelium is characterized by the formation of numerous long spirals 17 to 20 μ long, and 4 to 5 μ in diameter. Spherical and oval conidia formed abundantly, 1. to 1.5 x 1. to 1.2 μ . They stain readily, sometimes in a bi-polar manner.

Gelatin. Liquefaction starts rapidly in 3 to 4 days at 15° to 17° C., with no pigment production; then it becomes slower, with the production of a deep brown pigment in the unliquefied portion. White aerial mycelium is produced.

Potato plug. Growth continuous, folded, raised above the potato, of a gray to brown color. White to gray aerial mycelium appears early. Color of potato becomes black.

Hab. This forms also a common and numerous group of soil organisms. It has been isolated repeatedly from the local soils; garden soil at a depth of 1 inch, orchard soil at 12 and 30 inches, timothy soil at 4 inches, and Oregon soil. Herbarium Nos. 66, 68, 70, 71, 84 and 89.

Act. Halstedii, n. sp.

Czapeck's Agar. Colony gray, translucent, 4 to 8 mm. in diameter when 7 days old. Centre of colony is dark, with a large hyaline margin. Surface smooth. Aerial mycelium appears at an early date; it is at first white, then gull-gray (Rdg. liii-c.g.). Reverse of colony is colorless, turning dark in the centre. Medium not discolored. Odor fairly strong. Microscopically, the gray aerial mycelium was found to consist of long, slender, and spreading filaments. Close spirals, 7 to 10 μ in diameter, are borne as branches of the filaments. Conidia are oval to rod-shaped, 1.2 to 1.8 x 1. to 1.2 μ , often occurring in chains; they show only polar staining.

Gelatin. Rapid liquefaction with the production of a brown pigment in the unliquefied portion.

Potato plug. Growth solid, folded, greatly raised above the potato, gray to brown in color. White aerial mycelium covers only tip of growth. Color of potato is changed to black.

Czapeck's solution. Growth consists of 1 to 2 mm. colonies on side of vessel and bottom.

Hab. This is a common subsoil organism, isolated repeatedly from the deeper soil layers, but not from the surface soil. Garden soil at depths of 12, 20 and 30 inches; orchard soil at 12, 20 and 30 inches. Herbarium Nos. 33, 56, 72, 77, 85.

Act. Fradii, n. sp.

Czapeck's Agar. Colony 2 to 4 mm. in diameter, colorless, thin, with a smooth surface. Aerial mycelium is produced early; it is a thick cottony mass of a sea-shell pink color (Rdg. xiv-11'-f), with white tufts of mycelium in many places. Reverse colorless. No soluble pigment produced. Odor weak. Aerial mycelium consists of thick, long, unbranched filaments, which become branched only when old. Conidia numerous, rod-shaped, .75 to 1.25 x 0.5 μ , of a sea-shell pink color.

Gelatin. Rapid liquefaction with no color production. White aerial mycelium is produced early.

Potato plug. Growth glossy, thick, of a zinc-orange color (Rdg. xv-13'). White to rose aerial mycelium. Color of plug not changed.

Czapeck's solution. Numerous minute colonies all through medium and on surface.

Hab. Isolated once from the adobe soil. Herbarium No. 55.

Act. roseus, Krainsky.

Czapeck's Agar. Colony 2 to 3 mm. in diameter, of a pale brownish vinaceous color (Rdg. xxxix-5'''-f). Growth of colony is limited. Aerial mycelium is of the same color as the colony, and is produced at an early date. Medium uncolored. No odor or odor very weak. The general characters of the organism coincide closely with those given by Krainsky. Microscopically, the species is characterized by the formation of numerous close spirals. Conidia formation takes place early; they are abundant and oval in shape, 1.5 to 2 x 1. to 1.2 μ .

Gelatin. Slow liquefaction of the gelatin with the production of a deep brown pigment which spreads rapidly through the unliquefied portion of the gelatin.

Potato plug. Gray-yellow continuous streak on the plug. White aerial mycelium covers only tip of growth. Color of potato turns brown.

Czapeck's solution. Heavy, flaky mass all through liquid and on surface.

Glucose solution. Pink ring on the surface close to the glass. Some growth on the bottom.

Hab. Isolated from garden soil at depths of 8 and 12 inches. Herbarium Nos. 27 and 73.

Act. lavendulae, n. sp.

Czapeck's Agar. Colony 3 to 4 mm. in diameter, colorless, growing deeply into the medium in the form of long, colorless strands, with very little surface growth. Aerial mycelium appearing early in centre of colony; it is deep vinaceous lavender (Rdg. xlv-65'''-d), with a large sterile margin. A strong odor is present. Microscopically, close spirals were found, 5 to 8 μ in diameter. Conidia abundant, oval, 1.6 to 2. x 1. to 1.2 μ .

Gelatin. Slow liquefaction with the production of a brown pigment only after 6 days.

Potato plug. Golden brown, wide, thin, continuous growth. Color of potato black.

Hab. Isolated once from orchard soil at a depth of 4 inches. Herbarium No. 69.

Act. purpurogenus, n. sp.

Czapeck's Agar. Colony gray-translucent, 3 to 6 mm. in diameter, radially much wrinkled. Centre of colony is elevated, and colony itself becomes lichnoid in appearance. Centre is covered by white to purplish aerial mycelium, shading into dark grayish lavender (Rdg. xliii-57'''-C). Reverse of colony brownish to dark brown. No soluble pigment produced. Odor weak. Aerial mycelium is found under the microscope to be dense and twisted. Conidia oval, 1. to 1.5 x 0.8 to 1. μ .

Gelatin. Slow liquefaction with purplish coloration. White aerial mycelium is produced at an early date.

Potato plug. Gray-dark, much folded, continuous growth, with a pearly lustre. It looks as if many small individual colonies were massed together to form the streak. White aerial mycelum appearing late. Color of potato turned black.

Czapeck's solution. Large, thin, radiating colonies, rarely through medium and on surface.

Hab. Isolated repeatedly from the garden soil at depths of 20 and 30 inches, and orchard soil at 20 inches. Herbarium Nos. 59, 65 and 90.

Besides the classified organisms, several more cultures of unidentified species are at hand. Most of these develop very slowly, and not enough data could be collected for any grouping. They are kept for further study.

TABLE IV.
TABULAR STATEMENT OF SALIENT FEATURES OF ACTINOMYCES.

Name of Organism	Growth on Czapek's Agar				Gelatin (15% in dist. H ₂ O)			Liquid Czapek	Dextrose Broth 1%	Potato Plug			Odor
	Color of Colony	Aerial Mycelium	Reverse	Medium Colored	Liquefaction	Color of Medium	Aerial Mycelium			Color of Colony	Aerial Mycelium	Plug Colored	
<i>Act. violaceus-ruber</i>	red and blue	gray	red and blue	blue	slow	none	present white	flaky	surface	gray	white	blue	weak
<i>Act. violaceus-Caeseri</i>	gray	white	blue	plum-purple	rapid	none	none	flaky	bottom flaky	yellowish	scant, white	not colored	weak
<i>Act. violaceus-niger</i>	gray-black	white	black	black	rapid	none	none	colonies	brown	white	black	medium
<i>Act. erithro-chromogenus</i>	brown	white	brown	brown	slow	brown	present white	colonies	scant growth flaky	yellow-gray	none	black	strong
<i>Act. diastato-chromogenus</i>	gray	white	brown	brown	rapid	brown	none	flaky	gray	white	black	medium
<i>Act. purpureo-chromogenus</i>	brown	gray purple	purple	brown	slow	brown	none	flaky	orange brown	none	not colored	none
<i>Act. virido-chromogenus</i>	green-brown	black white	black	black	rapid	brown	none	green	white	black	medium
<i>Act. chromogenus (group)</i> ...	brown	white	black	brown	rapid	brown	none	brown	white	black	medium
<i>Act. exfoliatus</i>	brown	white	black	not colored	slow	none	present	colonies	gray-yellow	none	not colored	very weak
<i>Act. diastaticus</i>	gray	drab	dark	not colored	rapid	none	present	flaky	ring	gray-white	white	dark	weak
<i>Act. albus</i>	gray	gray	gray	not colored	rapid	brown	present	colonies	ring	brown	white-gray	not colored	weak
<i>Act. alboatrus</i>	colorless	white	brown	not colored	rapid	none	none	flaky & colonies	white	rosy	not colored	strong
<i>Act. reticuli</i>	colorless	white	creamy	not colored	rapid	brown	none	brown	white	dark	weak
<i>Act. citreus</i>	olive	gray	gray	not colored	rapid	none	white	flaky	little growth	gray	white & yellow	not colored	weak
<i>Act. flavus</i>	yellow	drab	yellow to olive-yel.	not colored	slow	brown	none	flaky	flaky	black	white	not colored	none

TABLE IV—(Continued).
TABULAR STATEMENT OF SALIENT FEATURES OF ACTINOMYCES.

Name of Organism	Growth on Czapek's Agar			Gelatin (15% in dist. H ₂ O)		Liquid Czapek	Dextrose Broth 1%	Potato Plug		
	Color of Colony	Aerial Mycelium	Reverse	Medium Colored	Liquefaction	Color of Medium	Aerial Mycelium	Color of Colony	Aerial Mycelium	Plug Colored
<i>Act. parvus</i>	yellow	yellow	brown	not colored	slow	none	none	gray	white	black
<i>Act. griseus</i>	olive buff	water-green	brownish	not colored	rapid	none	present	yellow & gray	white-gray	brownish
<i>Act. alboflavus</i>	yellowish	white	yellowish	not colored	rapid	none	none	gray	white	not colored
<i>Act. Verne</i>	Isabella color	yellow-brown	dark	green	rapid	none	none	yellow	scant, white	colored
<i>Act. albosporus</i>	Acajou red	white	red	not colored	rapid	none	present	gray	white	colored
<i>Act. Bobili</i>	red	none	red	not colored	rapid	brown	none	red	none	colored
<i>Act. Californicus</i>	red	gray	red	colored	slow	(late) none	present	red	none	colored
<i>Act. Lipmanii</i>	colorless	gray	dark	not colored	slow	none	none	red	none	colored
<i>Act. Rutgersensis</i>	colorless	gray	white	not colored	rapid	none	white-gray	ring	colored
<i>Act. Halsteadii</i>	gray to dark	white	colorless	colored	rapid	none	none	gray	white	not colored
<i>Act. aureus</i>	colorless	gray	white to brownish	colored	rapid	brown	present	brown	white	black
<i>Act. Fradii</i>	colorless	sea-shell pink	creamy	colored	rapid	none	white	orange	white	black
<i>Act. roseus</i>	rose	rose	light rosy	colored	rapid	brown	present	gray	rose	not colored
<i>Act. lavendulae</i>	colorless	lavender	creamy	colored	slow	brown	none	brown	white	brown
<i>Act. purpureogenus</i>	gray	gray to lavender	brown	brownish	slow	purplish	white	dark	white	black

KEY TO THE IDENTIFICATION OF THE ACTINOMYCES.

A. Gelatin liquefied rapidly, with no pigment produced in the unliquefied portion:**I. Spirals produced in the aerial mycelium:**

1. No pigment produced in the substratum, *Act. Rutgersensis*
2. Pigment produced in the substratum:
 - (a) Pigment dark blue, *Act. violaceus Caeseri.*
 - (b) Pigment brown, *Act. diastalicus.*

II. No spirals produced in the aerial mycelium:

1. No pigment produced in the substratum:
 - (a) Colony orange-red, aerial mycelium white, *Act. albosporus.*
 - (b) Colony rose-colored, aerial mycelium rosy, *Act. Fradii.*
 - (c) Colony a mixture of white and yellow:
 - (c') No conidia observed, *Act. albo-flavus.*
 - (c'') Conidia present in abundance:
 - x Conidia rod-shaped, colony powdery, gray-yellow. *Act. griseus.*
 - y Conidia spherical and oval, colony compact, citron-yellow. *Act. citreus.*
 - (d) Colony at first colorless, then becoming brown, to almost black:
 - (d') Aerial mycelium white, no conidia observed, *Act. albotratus.*
 - (d'') Aerial mycelium dark gray; conidia abundant, oval, *Act. Lipmanii.*
2. Pigment produced in substratum:
 - (a) Color of substratum green, *Act. Verne.*
 - (b) Color of substratum dark blue, *Act. violaceus-niger.*

B. Gelatin liquefied rapidly with the production of a brown pigment in the unliquefied portion:**I. Spirals produced in the aerial mycelium:**

1. Colony rose-colored, with rosy aerial mycelium, *Act. roseus.*
2. Colony colorless, with golden brown aerial mycelium, *Act. aureus.*
3. Colony slightly brown, with white aerial mycelium, *Act. Halstedii.*

II. Spirals not produced in the aerial mycelium:

1. No pigment produced on the agar substratum:
 - (a) Colony red to red-orange, with no aerial mycelium, *Act. Bobili.*
 - (b) Colony white, with white aerial mycelium:
 - (b') Aerial mycelium thin, rare, net-like, *Act. reticul.*
 - (b'') Aerial mycelium thick, white to gray, *Act. albus.*
2. Brown pigment produced in the agar:
 - (a) White aerial mycelium produced early and abundant, *Act. diastato-chromogenus.*
 - (b) White aerial mycelium not produced at all, or very late, *Act. chromogenus group.*
 - (c) Surface of colony green, white aerial mycelium produced early, *Act. virido-chromogenus.*

C. Gelatin slowly liquefied, with no pigment production:

I. Spirals produced in the aerial mycelium:

1. Production of soluble red and blue pigments, *Act. violaceus-ruber*.
2. No soluble pigment produced; red mycelium grows deep into the substratum, *Act. Californicus*.

II. No spirals produced in the aerial mycelium:

1. No pigment produced in the substratum, colony yellow, *Act. parvus*.
2. Brown pigment produced, colony tends to crack, *Act. exfoliatus*.

D. Gelatin slowly liquefied, with the production of a brown pigment:

I. Spirals produced: aerial mycelium lavender color, *Act. lavendulae*.

II. No spirals produced in the aerial mycelium:

1. Colony yellow, aerial mycelium gray, *Act. flavus*.
2. Colony colorless, aerial mycelium white-purplish, *Act. purpurogenus*.
3. Colony black, lichnoid, aerial mycelium scant, *Act. erithrochromogenus*.
4. Colony purple, no aerial mycelium, *Act. purpochromogenus*.

PHYSIOLOGY OF THE ACTINOMYCES.

Investigators find that most of these organisms grow best at 30° C. The minimum lies near 15° C. and the maximum is about 50° C.

The cellulose destroying power of the actinomyces has been studied by Krainsky (13), Scales (22), and several others.

As to their ability to liberate ammonia, Lutman and Cunningham (15) by inoculating nutrient broth with *Act. chromogenus* found 10 to 20 mg. of NH_3 in 50 c.c. of culture for from 14 to 30 days. Since liberation of ammonia from organic compounds is one of the most important factors in the study of organisms from the soil fertility standpoint, a series of experiments were started for the purpose of demonstrating the part played by the actinomyces in this process.

One hundred grams of soil and 2.42 gm. of cottonseed meal containing 155 mg. of nitrogen were placed in Erlenmeyer flasks and well mixed. Twenty cubic centimeters of water (equal to 70 per cent saturation for the soil used) were added. Two sets of flasks received 10 c.c. each and another two 40 c.c. each. Flasks were plugged and sterilized in the autoclave, 15 minutes at 15 pounds pressure. Cultures of the different organisms grown on Czapeck's solution were used for inoculation, 1 c.c. being added. Duplicates were used throughout. After 7, 14 and 30 days, respectively, the ammonia of the various sets was distilled off with MgO in the usual way. Table IV shows the results of the investigation.

One can readily see from Table V that the actinomyces do not play any appreciable rôle in the soil as ammonifiers, as judged by ammonification in soil sterilized by steam under pressure. Only six organisms gave more than 1 mg. of nitrogen as NH_3 , all the rest accumulating less than that amount in 14 days. When the incubation period was extended to 30 days somewhat larger quantities of NH_3 were found to have been

TABLE V.
AMMONIA ACCUMULATION BY ACTINOMYCES IN THE SOIL.

Name of Organism	Period of Incubation	Moisture Content	Mg. of N. as Ammonia	Mg. of N. Average	Mg. of N. Minus check
Act. violaceus-ruber	14 days	20%	4.06 4.36	4.21	0.51
Act. violaceus-Caeseri	14 days	20%	3.64 4.10	3.87	0.17
Act. roseus	14 days	20%	3.52 3.06	3.29	-0.41
Act. erythrochromogenus	14 days	20%	4.06 4.67	4.37	0.67
Act. griseus	14 days	20%	4.71 4.10	4.41	0.71
Act. griseus	14 days	10%	3.33 3.46	3.40	-0.30
Act. griseus	14 days	40%	7.01 7.16	7.09	3.39
Act. griseus	30 days	20%	9.18 9.84	9.51	5.81
Act. albus	7 days	20%	4.56 4.12	4.34	0.74
Act. albus	14 days	20%	4.24 4.88	4.56	0.86
Act. albus	14 days	10%	3.16 3.94	3.55	-0.15
Act. albus	14 days	40%	7.01 7.63	7.32	3.62
Act. albus	30 days	20%	19.2 19.8	19.5	15.8
Act. citreus	14 days	20%	3.80 3.80	3.80	0.10
Act. flavus	14 days	20%	3.75 4.06	3.91	0.21
Act. albosporeus	14 days	20%	4.56 4.25	4.41	0.71
Act. albo-flavus	14 days	20%	3.75 3.75	3.75	0.05
Act. Lipmanii	14 days	20%	4.36 4.88	4.62	0.92
Act. Chromogenus (No. 22)	14 days	20%	4.51 3.75	4.13	0.43

accumulated. When the different moisture contents employed are compared it is found that the highest moisture content gave the highest ammonia accumulation.

It has been found that the actinomyces readily assimilate NO_2 , NO_3 , NH_3 , and organic compounds of nitrogen. The characteristic point is that they reduce nitrate to nitrite, but not to free nitrogen or ammonia.

Not all actinomyces produce an odor, but as all representatives of the *Act. chromogenus* group seem to have an odor, it was formerly believed that this property was characteristic of the whole genus. The odor, when present, varies from very strong to very weak. The stronger odors are suggestive of a musty straw stack, and some of the milder ones of the smell of soil.

Heinze (11) suggested that the actinomyces probably play an important part in the formation of humus out of the organic matter of the soil, even when the soil has an acid reaction. Fousek (18) found that "streptothrix" do not nitrify; on the contrary, they reduce nitrates to nitrites, but without loss of free nitrogen. In view of the ready assimilation of nitrates, ammonia compounds, urea, and uric acid, by these organisms, it seems possible that they may help to "fix" nitrogenous fertilizers in the soil and prevent their being lost by leaching or denitrification. He (Fousek) states that the "streptothrix" have a favorable influence upon plant growth, because through their rapid decomposition of the organic matter, plant nutrients are set free and made available for the higher plants.

The knowledge that the actinomyces are strong cellulose decomposers and weak producers of ammonia leads one to think that the probable rôle of the organism in the fertility of the soil lies in the formation of humus. Organic matter when applied to the soil has to undergo a series of processes before it can be utilized by the higher plants. Among the most important of these is the decomposition of cellulose, and subsequent formation of humus. In this process the actinomyces probably play an important, if not a dominant part, together with the cellulose decomposing bacteria and fungi.

In arid soils where cellulose destruction has been found to be extremely rapid, we should, therefore, expect to find actinomyces in abundance. That this might be the case is indicated by the figures in Table III, representing the soils from southern California.

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PLATE I.

- Fig. 1—Act. violaceus-ruber.
- Fig. 2—Act. violaceus-Caeseri.
- Fig. 3—Act. chromogenus, strain 22.
- Fig. 4—Act. virido-chromogenus.
- Fig. 5—Act. diastato-chromogenus.
- Fig. 6—Act. erithro-chromogenus.
- Fig. 7—Act. purpeo-chromogenus.
- Fig. 8—Act. chromogenus, strain 40.



Fig. 1.



Fig. 2.



Fig. 3.

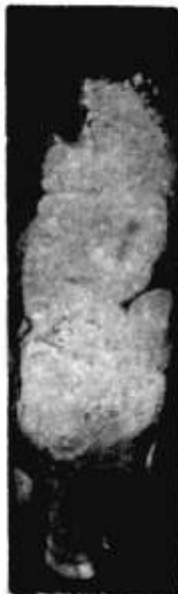


Fig. 4.



Fig. 5.



Fig. 6.



Fig. 7.



Fig. 8.

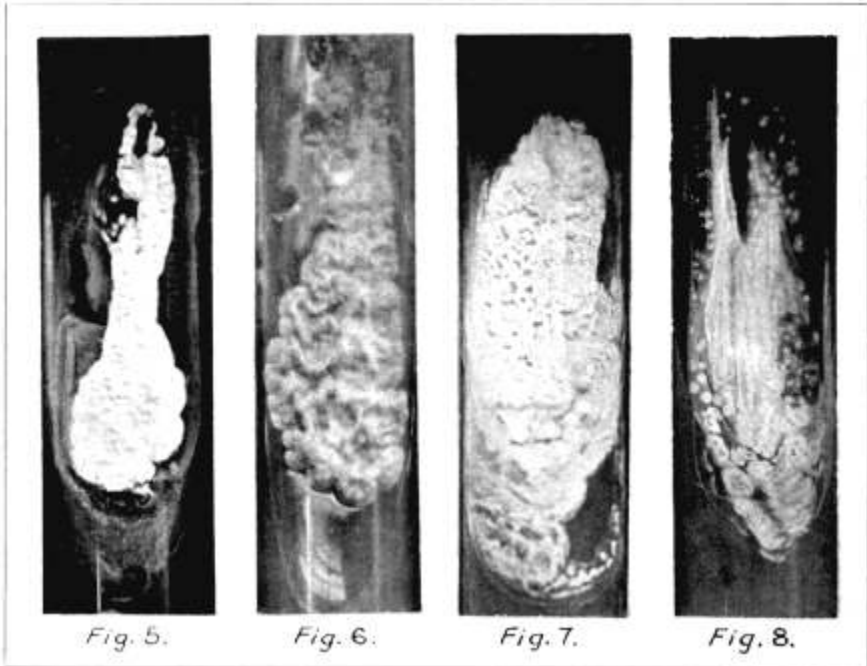
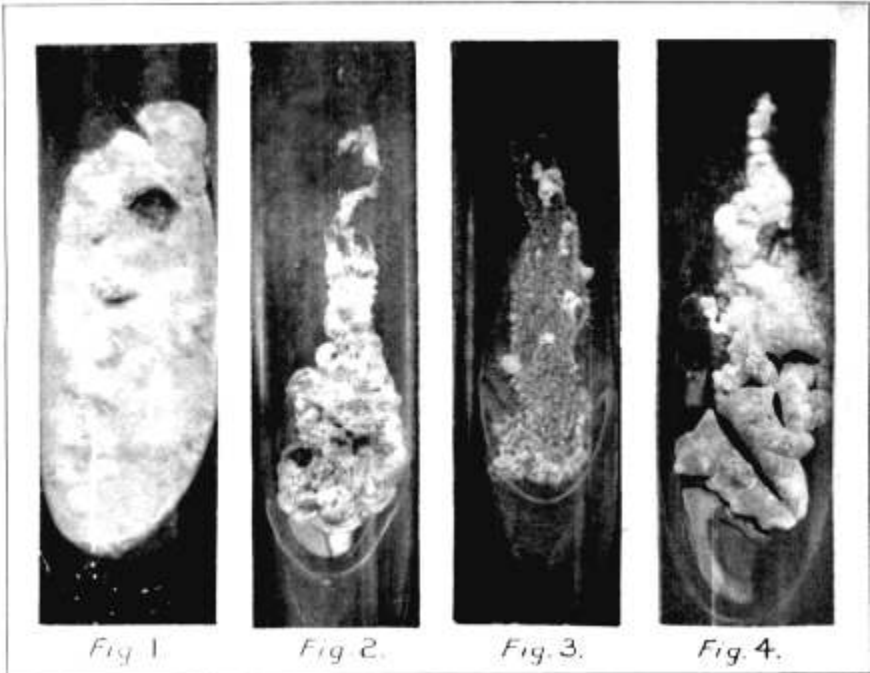


PLATE II.

- Fig. 1—Act. albus.
- Fig. 2—Act. albotratus.
- Fig. 3—Act. reticuli.
- Fig. 4—Act. alboflavus.
- Fig. 5—Act. albosporeus.
- Fig. 6—Act. Verne.
- Fig. 7—Act. griseus.
- Fig. 8—Act. Californicus.

PLATE III.

- Fig. 1—Act. citreus.
- Fig. 2—Act. Bobili.
- Fig. 3—Act. purpurogenus.
- Fig. 4—Act. Lipmanii.
- Fig. 5—Act. diastaticus.
- Fig. 6—Act. Fradii.
- Fig. 7—Act. exfoliatus.
- Fig. 8—Act. flavus.
- Fig. 9—Act. Rutgersensis.
- Fig. 10—Act. roseus.

