FRESH ISOLATES OF ACTINOMYCETES IN WHICH THE PRESENCE OF SPOROGENOUS AERIAL MYCELIA IS A FLUCTUATING CHARACTERISTIC[1, 2]

KENNETH L. JONES

Department of Botany, University of Michigan, Ann Arbor, Michigan

Received for publication October 25, 1948

Actinomycetes of saprophytic, aerobic habit have vegetative mycelia of true-branching hyphae that grow in and on the substratum. Aerial mycelia composed of more or less erect filaments may arise within 2 to 30 days on a new growth. The filaments are usually coarser than those of the vegetative mycelium, and they are more apt to be acid-fast (Umbreit, 1939), perhaps because of the lipid character of the outer wall (Erickson, 1947*a*). Spores may be produced by the aerial hyphae. They have been termed conidia, although their origin may be endogenous. The species that are especially abundant in the soil form chains of aerial spores and were hence named *Streptomyces* by Waksman and Henrici (1943). The species that lack conidia but have a vegetative mycelium that fragments were placed in the genus *Nocardia*.

The presence of aerial spores is unfortunately not a fixed characteristic, partly because the presence of aerial mycelia is variable. In fact, in *Nocardia* (*Proactinomyces* of Jensen) "the property of forming aerial mycelia seems easily lost" (Jensen, 1931), and it "cannot actually be used as a criterion for separation, since it is dependent to a large extent, on the cultural conditions" (Umbreit, 1939). In *Streptomyces* the loss may be permanent (Appleby, 1948) or temporary (Schatz and Waksman, 1945). If permanent, it occurs suddenly as a mutation or gradually from continued culture on artificial media. The temporary loss is a direct effect of the cultural conditions, a genetically controlled fluctuation, or an interaction of the two.

If a culture of *Streptomyces* fails to produce aerial hyphae, it is indistinguishable from species of *Nocardia* that have a persistent vegetative mycelium, *β-Proactinomyces* of Umbreit (1939). The occurrence of such cultures in *Streptomyces griseus* prompted Schatz and Waksman (1945) to raise the question, "To what extent may many of the species of *Nocardia* described in the literature represent variants of *Streptomyces* species that have lost the property of producing aerial mycelium?" The question, as these authors indicated, is not merely one of nomenclature. The variants lacking aerial mycelia may be quite unlike the original in biochemical and morphological properties. In industrial microbiology this may have reference to enzymes, antibiotics, and acids, as well as to the physical nature of the growth en masse. In the soil it is likely that growths lacking spores are more restricted in their distribution than are the sporeformers and that they differ from them in attacking plant residues.

[1] Paper number 885 from the Department of Botany of the University of Michigan.

[2] This work was aided by a grant from the Horace H. Rackham School of Graduate Studies.

The frequency of variants lacking aerial mycelia, either temporarily or permanently, is unknown. Schaal (1944) found that, in the actinomycetes producing potato scab, sectoring is especially associated with certain strains. Skinner (Skinner, Emmons, and Tsuchiya, 1947) believes that "although variant sectors are sometimes seen, they do not seem to occur more frequently than they do in colonies of molds and bacteria of equal age." The effect of the medium on the presence or absence of aerial hyphae has in a general way been commented on by several workers, as has the physiological degeneration, including the loss of sporulation. Jones (1946) and Erikson (1947*b*) have found that sporulation may be restored by returning the organisms to soil; in fact, sterilized soil is an excellent medium for maintaining stocks of *Streptomyces*.

The writer (1940) became interested in fluctuating variations that occurred in monosporic cultures, e.g., strain 47–13, that failed to form aerial growth after only a few transfers, but that later from time to time regained the property in a most unpredictable manner. Chance observations of this phenomenon in other fresh, vigorous isolates and in culture collections of *Nocardia* led to the present attempt to get some idea of its frequency.

MATERIAL AND METHODS

A representative collection of fresh isolates of *Streptomyces* was obtained from a wide range of soils in the United States. I am indebted to Professor F. K. Sparrow, Jr., for the use of his soil collections from Bikini, Java, Sumatra, and Brazil; to Thomas Muzik for soils from North Africa; and to Margery Anthony for samples collected in Mexico.

Soil suspensions were prepared in sterile tap water and plated in Jensen's (1930) sodium caseinate, glucose agar. After two weeks representative colonies of *Streptomyces* bearing a heavy felt of sporogenous, aerial growth were selected for transfer to Bennett's medium, which was the most favorable of those tested for the production of aerial mycelia. The formula for this medium was worked out by Dr. Ralph Bennett, who has kindly consented to its inclusion here.

Bennett's medium for sporulation in Streptomyces

Yeast extract	1.0 g
Beef extract	1.0 g
"N-Z-amine A"[3]	2.0 g
Glucose	10.0 g
Distilled water	1,000.0 ml
Adjust to pH 7.3 with NaOH	

The other agar media tested were Czapek's, modified Czapek's with glycerol substituted for sucrose, Krainsky's glucose asparagine, glycerol nutrient, starch, and calcium malate. Bennett's medium usually gave the heaviest growth of aerial mycelia, although as Carvajal (1946) stated, "The best medium for spore production for one strain may be unsatisfactory for another."

On being transferred, the aerial mycelium was inoculated on plates of solid agar at 25 successive points, 1.5 cm apart. This permits full development of

[3] "N-Z-amine A" is an enzymatic hydrolyzate of casein.

colonies and brings out any variation which may result from the amount of the inoculum (Jones, 1946). There were cultured 1,298 isolates, with results in the first plating as follows: 1,045, or 80.5 per cent, produced only colonies with aerial mycelia; 173, or 13.3 per cent, formed some colonies with and others without aerial mycelia; and 80, or 6.1 per cent, produced only colonies that lacked aerial mycelia.

Sixty-three of the isolates that were in the group lacking aerial mycelia were transferred to fresh plates at monthly intervals. A summary of the results of the first 6 transplants is given in table 1, where they are designated as group I. Several of the isolates were not able to survive on laboratory media. They usually formed soft, pasty colonies of fragmented or autolyzed mycelia. Seven of the vigorous isolates produced only colonies with vegetative growth; however, 3 of these have since given rise to colonies with aerial mycelia bearing spores.

TABLE 1

A summary of the presence or absence of aerial mycelia in two groups of "Streptomyces"

(Group I isolates lacked aerial mycelia in the first transfer after isolation from soil, whereas group II possessed aerial mycelia on all colonies. The isolates were grown as colonies, 25 per plate, on Bennett's medium and transferred once a month for 6 months.)

GROUP	NO. OF ISOLATES	NO. OF ISOLATES TO SURVIVE 6 TRANSFERS	AERIAL MYCELIA PRESENT	AERIAL MYCELIA (±)	AERIAL MYCELIA ABSENT
I	63	45	5 (11%)	33 (73%)	7 (15%)
II	96	92	85 (92%)	7 (7%)	0 (0%)

"Aerial mycelia present" indicates that every colony in the 6 transfers possessed them. "Aerial mycelia (±)" indicates that some colonies lacked aerial mycelia and others possessed them. "Aerial mycelia absent" indicates that every colony in the 6 transfers lacked them.

Thirty-three (73 per cent) formed aerial growth on some colonies but not on others, and 5 produced aerial filaments on every colony. For comparison with group I, 96 isolates were selected from the 1,045 that possessed aerial mycelia on all colonies in the first plating on Bennett's medium, and these, designated as group II, were transferred similarly at monthly intervals. Table 1 indicates that the isolates in group II generally survived and were much more stable with reference to the production of aerial mycelia than were those of group I.

The divergent behavior of the two groups was maintained when the isolates were afterwards grown on Krainsky's glucose asparagine agar and on Czapek's medium. In group I, 33 per cent formed colonies every one of which possessed a heavy felt of sporogenous hyphae on Krainsky's medium, whereas 90 per cent of group II showed this property. On Czapek's medium, 15 per cent of group I were sporogenous as compared with 78 per cent for group II. Some isolates gave positive results on one medium and not on another; however, this was not the general rule. Jones (1946) and Erikson (1947*b*) have emphasized the influence of the penultimate medium. It is likely that certain isolates would have given different results had they been grown first, say, on Krainsky's and then on Bennett's medium.

DISCUSSION

Streptomyces is designated by Waksman and Henrici (1943) as the genus of actinomycetes in which aerial spores ("conidia") are produced in chains. These investigators and their associates, however, have been fully aware of the labile nature of sporulation. Schatz and Waksman (1945) showed that asporous variants of *Streptomyces griseus*, under certain conditions of culture, reverted to the parent sporulating strain. In *Henrici's Molds, Yeasts, and Actinomycetes* (Skinner, Emmons, and Tsuchiya, 1947, p. 357) it is stated, "It is not an uncommon experience to find conidia develop rather suddenly on a strain which has not shown any aerial mycelium during many months of subcultivation, and old laboratory strains, which formed conidia when first isolated, frequently have a tendency to lose the ability to form conidia regularly."

It is debatable whether or not the aerial mycelium and its spores represent a stage in the life cycle of *Streptomyces* that is fundamentally unlike the vegetative mycelium. Badian (1936) and Klieneberger-Nobel (1947) believe that the two are distinct: the spores are a diploid stage and the vegetative mycelium is haploid. Badian thinks that a union of chromosomelike bodies occurs in the aerial hyphae and the resultant diploid spores undergo reduction division on germination. Klieneberger-Nobel believes there is a fusion of filaments in the primary mycelium to form initial cells "which consist of darkly staining nuclear bodies surrounded by cytoplasm and later enclosed by a cell wall." The initial cells produce the aerial or secondary mycelium.

Whether spores are haploid or diploid, or even possess chromosomes, it has been proved that they may form in the vegetative filaments as well as in the aerial ones. Krassilnikov (1938) observed and illustrated spores in his *Proactinomyces ruber* n. sp., an organism ordinarily lacking aerial growth. "The spores are formed by the breaking up of the cell plasma into separate portions usually into 3 to 5; every portion becomes rounded, covered with a membrane and transforms into a spore, the membrane of the mother cell dissolves and disappears. The spores germinate in the same way as those of actinomyces, they form germs which develop into a mycelium." Carvajal (1947) observed and clearly photographed spores in the vegetative filaments of *Streptomyces griseus*, *Streptomyces lavendulae*, *Streptomyces albus*, and an unidentified chromogenous species.

The natural distribution of *Streptomyces* species must depend primarily on the ability to sporulate abundantly and regularly. Jones (1944) found that cultures "in sterilized soil differ markedly in their sporulation." Some cover the surface of the soil with a heavy powder of conidia; in others there is no macroscopic evidence of spores.

The actinomycetes in which the formation of aerial spores is a fluctuating characteristic present a taxonomic dilemma. Are they *Nocardia* or *Streptomyces*? It is not known whether the isolates in nature were asporous or sporogenous. The behavior on laboratory media is too irregular to show whether they are acquiring or losing the property of sporulation. It is true that most of the vigorous ones produce colonies like those of *Streptomyces* (or *β-Proactinomyces* of Umbreit, 1939), in which the stable mycelium forms a leathery stroma.

However, some are bacterialike, with the mycelium fragmenting or disintegrating to give pasty colonies.

SUMMARY

Colonies of 1,298 isolates of *Streptomyces* were grown from fresh isolates of soil organisms obtained from the United States, Mexico, South America, Africa, and the South Pacific. In one-fifth of the isolates the characteristic of aerial mycelia was a fluctuating one from the start. Six per cent formed only vegetative growth in the first transfer. However, even these proved to fluctuate on further subcultivation. A representative sample of the four-fifths that formed only sporogenous colonies in the first plating remained relatively stable on subcultivation. The assignment of the fluctuating group to *Streptomyces* or to *Nocardia* would be entirely arbitrary.

REFERENCES

APPLEBY, J. C. 1948 An asporogenous variant of *Streptomyces griseus*. J. Gen. Microbiol., **2**, 80–82.

BADIAN, J. 1936 Ueber die zytologische Struktur und den Entwicklungszyklus der Actinomyceten. Acta Soc. Botan. Polon., **13**, 105–126.

CARVAJAL, F. 1946 Biological strains of *Streptomyces griseus*. Mycologia, **38**, 596–607.

CARVAJAL, F. 1947 The production of spores in submerged cultures by some *Streptomyces*. Mycologia, **39**, 426–440.

ERIKSON, D. 1947*a* Differentiation of the vegetative and sporogenous phases of the actinomycetes. 1. The lipid nature of the outer wall of the aerial mycelium. J. Gen. Microbiol., **1**, 39–44.

ERIKSON, D. 1947*b* Differentiation of the vegetative and sporogenous phases of the actinomycetes. 2. Factors affecting development of the aerial mycelium. J. Gen. Microbiol., **1**, 45–52.

JENSEN, H. L. 1930 Actinomycetes in Danish soils. Soil Sci., **30**, 59–77.

JENSEN, H. L. 1931 Contributions to our knowledge of the *Actinomycetales*. II. The definition and subdivision of the genus *Actinomyces*, with a preliminary account of Australian soil actinomycetes. Proc. Linn. Soc. N.S.W., **56**, 345–370.

JONES, K. L. 1940 Colony variation in actinomycetes under constant environmental conditions. Soil Sci. Soc. Am. Proc., **5**, 255–258.

JONES, K. L. 1944 The influence of soil depth upon distribution of actinomycetes. Papers Mich. Acad. Sci., **29**, 15–22.

JONES, K. L. 1946 Further notes on variation in certain saprophytic actinomycetes. J. Bact., **51**, 211–216.

KLIENEBERGER-NOBEL, E. 1947 The life cycle of sporing *Actinomyces* as revealed by a study of their structure and septation. J. Gen. Microbiol., **1**, 22–37.

KRASSILNIKOV, N. A. 1938 *Proactinomyces*. Bull. acad. sci. U.S.R.S., 139–172. English summary, 171–172.

SCHAAL, L. A. 1944 Variation and physiological specialization in the common scab fungus (*Actinomyces scabies*). J. Agr. Research, **69**, 169–187.

SCHATZ, A., AND WAKSMAN, S. A. 1945 Strain specificity and production of antibiotic substances. IV. Variations among actinomycetes, with special reference to *Actinomyces griseus*. Proc. Natl. Acad. Sci. U. S., **31**, 129–137.

SKINNER, C. E., EMMONS, C. W., AND TSUCHIYA, H. M. 1947 Henrici's molds, yeasts, and actinomycetes. 2d ed. John Wiley and Sons, New York.

UMBREIT, W. W. 1939 Studies on the *Proactinomyces*. J. Bact., **38**, 73–89.

WAKSMAN, S. A., AND HENRICI, A. T. 1943 The nomenclature and classification of the actinomycetes. J. Bact., **46**, 337–341.