AGAR-DECOMPOSING STRAINS OF THE ACTINOMYCES COELICOLOR SPECIES-GROUP

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The ability to decompose agar has not hitherto been recorded for any member of the Actinomycetales, and consequently it seemed desirable to make a detailed study of a typical Actinomyces possessing this property, which was discovered fortuitously. The organism appeared on a discarded tapwater agar plate (used originally for the germination of myxomycete sclerotia) in the form of blue-black colonies with scanty greyish-white aerial mycelium, each of which lay in the center of a marked depression in the agar. Several strains were isolated and purified by repeated streaking on mineral agar plates with \((NH_4)_2SO_4\) as a nitrogen source. The behavior of the pigment produced (blue under basic, red under acid conditions) marked the organism at once as a member of the widespread soil group of litmus actinomycetes, which are classified in Bergey’s Manual under the name \(A.\ coelicolor\).

In response to my request, Dr. S. A. Waksman very kindly provided me with an authentic culture of this species from his collection, together with several agar-decomposing strains which had been isolated at the New Jersey Agricultural Experiment Station in the course of other work. These cultures (NB strains) together with my own isolates (PG strains) comprised the material for the following studies.

MEDIA AND METHODS

The medium used for the maintenance of stock cultures and for the studies on variation consisted of \((NH_4)_2SO_4\) 0.1 g., \(K_2HPO_4\) 0.1 g., \(MgSO_4\) 0.02 g., \(NaCl\) 0.01 g., \(CaCl_2\) 0.01 g., \(FeCl_3\) 0.002 g., Bacto agar 2.0 g., distilled water 100 ml., pH adjusted to 7.0–7.3. Occasionally the \((NH_4)_2SO_4\) was replaced by \(KNO_3\). The former medium becomes acid, the latter basic, as a consequence of growth, with resultant differences in the color of the pigment, but all other characters of the organisms are identical on both media.

The utilization of carbon sources was studied in liquid media with the mineral base given above (\(KNO_3\) as N-source) and the various substrates to be tested in a concentration of 1.0 per cent.

For the demonstration of agar decomposition Gran’s test (flooding the plates with an I-KI solution) was used, although in most cases the gelase fields were already evident without this treatment.

Cultures were incubated at 28°C. In the work on variation, plates were

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always compared and photographed after 4 days at 28°C, followed by 4 days at room temperature, because it was found that an exposure to the lower temperature often resulted in a marked intensification of pigment production and consequent better differentiation of the variant types.

**Variability of the agar-decomposing strains**

The extreme variability of actinomycetes is a well-known phenomenon, first clearly shown by the able studies of Lieske (1921). However, with the exception of the work of Krassilnikov and Tausson (1938) on *Proactinomyces* species, there has apparently been no detailed investigation since Lieske's time on the occurrence and mechanism of transmissible variations ("Mutationen" *sensu* Lieske) in the mycelial members of this order.

The litmus actinomycetes appear to be an extremely unstable group, a fact attested as early as 1913 by Beijerinck, whose important paper on this subject has been overlooked by most subsequent workers. He wrote of his isolates:

"Ich fand bei beinahe jeder Aussaat . . . nicht nur verschieden gestaltete Kolonien, sondern auch Sektormutanten, welche bei der Vermehrung entweder atavierten oder erblich stabile morphologische Typen hervorbrachten."

The agar-decomposing strains proved to be no exception to this rule, and since the criterion of agar-decomposition could be used as a check on the origin of the different variants, they provided good material for a re-examination of the subject.

At the time of isolation, a remarkable type of transient biochemical variation was observed. On first streaking the PG strains, only a few agar-decomposing colonies were obtained, interspersed between numerous (ca. 95 percent) apparently non-agar-decomposing ones. The former grew far more rapidly than the latter, were more brightly pigmented, and produced an extensive depression of the surrounding agar in 2–3 days. At first it was thought that the cultures contained two different kinds of actinomycetes, but on restreaking, both types gave rise to the preceding 95–5 per cent ratio of "non"-agar-decomposers to agar-decomposers. On these plates it was noted that after 7–10 days the "non"-agar-decomposing colonies had begun to attack the agar, although they never attained the size, pigmentation and gelase production of the initially agar-decomposing ones. Again both types were streaked; the "non"-agar-decomposers now began to attack agar in 4–5 days. The same treatment was continued through several more transfers with the ultimate result that the difference in the rate of agar decomposition, and with it the differences in colony aspect, disappeared.

From time to time during this work variant colonies were found which, although they attacked agar readily, differed from the normal type in pigmentation, size, or some other character. One of them (PG 3), a small, very intensely pigmented form with sparse aerial mycelium, yielded a bewildering variety of different types on subculture. Four colonies were selected for further study from among those which appeared on the plate.
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1. A small, deeply pigmented colony with no aerial mycelium.

2, 3 and 4. Very irregular colonies, sectored with respect to color and aerial mycelium.

Colony 1 gave an extremely uniform picture on subculture, all the colonies resembling the parent. This type (1a) is illustrated in fig. 2. The strain could be maintained in constant form through repeated subcultures provided that transfers were always made from the substrate mycelium. Type 1a was devoid of aerial mycelium in the younger stages, but an occasional colony would produce a scanty, irregularly sectored aerial mycelium with conidia after about a week, and almost every colony would do so after two or three weeks. If transfers were made from colonies in this condition an extreme variety resulted, most of the progeny producing conidia in abundance within a few days and bearing not the least resemblance to the parent types (figs. 3 and 4). A further analysis was not attempted.

In the subculture from colony 2 the majority of colonies were large, with an almost colorless or pale yellow substrate mycelium, producing a slight amount of a diffusible yellow pigment and an abundant white aerial mycelium bearing conidia. On ageing, the colonies produced the litmus pigment to a slight degree, the substrate mycelium and surrounding agar becoming green (blue

2 Except where otherwise stated, pigmentation refers to the litmus-like pigment.

Fig. 1. Plate culture of an unstable form (progeny of a 2B2 type variant), treated with I-KI solution to show the gelase fields.

All the colonies, despite their extreme dissimilarity, decompose agar. Figs. 2, 3, 4, 5, 7, 8, 10 and 11 are strictly comparable to fig. 1 with respect to the age of the cultures when photographed and the magnification of the prints.
Fig. 2. Variant 1a, treated with I-KI solution to show the gelase fields. Aerial mycelium begins to become visible on a few colonies.

Fig. 3. Variant 1a streaked from the substrate mycelium. Compare with Fig. 4.
litmus pigment plus yellow pigment) or pinkish orange depending on the pH. This type, designated as 2a, proved exceptionally stable through continued transfer, although occasionally one or two deeply pigmented colonies with little aerial mycelium would appear on a plate. Type 2a is illustrated in figs. 5 and 6.

Type 2b, represented by only a few colonies on the original colony 2 plate, was a small, heavily pigmented form with an irregularly sectored grey aerial mycelium. On subculture it gave rise to a large number of further variants, among which stable 2a was common; other well represented types were (1) large heavily pigmented colonies with no aerial mycelium (2b1), (2) typical litmus actinomycete colonies (large and intensely pigmented, the pigment diffusing into the surrounding agar, and with an abundant white aerial mycelium

---2b2) and (3) colorless, inconspicuous colonies deeply embedded in the agar and without aerial mycelium (2b3). Type 2b1 remained stable on subculture. Type 2b2 was highly unstable, and broke up at once into a large variety of forms among which 2a was common. Type 2b3 remained stable but died out after a few transfers owing to its tendency to undergo a very rapid autolysis. Type 2b3 is represented in fig. 7; figs. 1 and 8 shows examples of the type of subculture resulting from the streaking of 2b2.

Type 2c, like 2b, was represented by only a few colonies on the original plate of 2. The substrate mycelium was colorless or very faintly pigmented and deeply embedded in the agar without at first any aerial mycelium. Older colonies developed a scant aerial mycelium which was always completely devoid of conidia. Type 2c was notable for its remarkable manner of growth, which
was most clearly apparent in well-isolated colonies 3–4 weeks old. The substrate mycelium spread out extensively as an irregular web of hyphae in all directions below, but not on the surface of the agar. This has never been seen in any of the other variants. Type 2c remained stable on subculture.
FIG. 7. VARIANT 2B3, TREATED WITH I-KI SOLUTION TO SHOW THE GELASE FIELDS
Some of the colonies have begun to autolyze


Colony 3 resulted in a number of variant forms, of which 2a was common and remained, as before, stable on subculture. Type 2b also occurred extensively, giving much the same picture on subculture as previously. In addition a new
type (3a) appeared, which was characterized by its large maroon colonies either without or with very scanty (conidia-bearing) white aerial mycelium. The maroon pigment appeared to differ from the typical litmus pigment in that it
was retained within the substrate mycelium and never changed color with pH changes. Old cultures produced in addition a small amount of the litmus pigment, which diffused into the surrounding agar. On the whole, 3a was fairly stable but subcultures would split off a few 2b2 colonies, often with considerable sectoring of the aerial mycelium. As before, the 2b2 colonies were

highly unstable, giving rise to a number of different types, predominant among which was 2a. 3a is illustrated in fig. 9.

Colony 4 behaved similarly to 3. However, in addition to the variants found on 3, there appeared a few extremely small, colorless, inconspicuous colonies without any aerial mycelium which decomposed agar only to a very slight extent (4a). This type maintained its characters on subculture, but died out after only a few transfers due to its tendency to rapid autolysis (fig. 10). Except

TABLE 1
A comparison of some properties of the variants

<table>
<thead>
<tr>
<th>TYPE</th>
<th>COLONY SIZE</th>
<th>PIGMENTATION</th>
<th>AERIAL MYCELIUM</th>
<th>CONIDIA</th>
<th>TENDENCY TO AUTOLYSIS</th>
<th>STABILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Small</td>
<td>Much litmus pigment</td>
<td>White, scantly, delayed</td>
<td>Present</td>
<td>Absent</td>
<td>Stable except when transferred in conidial stage</td>
</tr>
<tr>
<td>2a</td>
<td>Large</td>
<td>Yellow pigment, very little litmus pigment</td>
<td>White, abundant</td>
<td>Present</td>
<td>Absent</td>
<td>Stable</td>
</tr>
<tr>
<td>2b</td>
<td>Small</td>
<td>Much litmus pigment</td>
<td>Grey, scantly, sectored</td>
<td>Present</td>
<td>Absent</td>
<td>Very unstable</td>
</tr>
<tr>
<td>2b1</td>
<td>Large</td>
<td>Much litmus pigment</td>
<td>None</td>
<td>Absent</td>
<td>Absent</td>
<td>Stable</td>
</tr>
<tr>
<td>2b2</td>
<td>Large</td>
<td>Much litmus pigment</td>
<td>White, abundant, often sectored</td>
<td>Present</td>
<td>Absent</td>
<td>Very unstable</td>
</tr>
<tr>
<td>2b3</td>
<td>Small</td>
<td>None</td>
<td>None</td>
<td>Absent</td>
<td>Considerable</td>
<td>Stable</td>
</tr>
<tr>
<td>2c</td>
<td>Large</td>
<td>Very small amount of litmus pigment</td>
<td>Scanty, delayed</td>
<td>Absent</td>
<td>Absent</td>
<td>Stable</td>
</tr>
<tr>
<td>3a</td>
<td>Large</td>
<td>Much maroon pigment, a little litmus pigment</td>
<td>Scanty, delayed</td>
<td>Present</td>
<td>Absent</td>
<td>Fairly stable</td>
</tr>
<tr>
<td>4a</td>
<td>Very small</td>
<td>None</td>
<td>None</td>
<td>Absent</td>
<td>Considerable</td>
<td>Stable</td>
</tr>
</tbody>
</table>
for the difference in size and gelase fields (compare figs. 7 and 10), 4a was very similar to 2b3. In table 1 some properties of the variants have been summarized and compared.

NUTRIENT REQUIREMENTS AND CULTURAL CHARACTERISTICS OF THE DIFFERENT STRAINS

The following nine strains were used: NB 3442, NB 3443, NB 3445, NB 3355 (authentic non-agar-decomposing culture of \( A. \ coelicolor \)), PG 1, PG2, PG3 variant 1a, PG 3 variant 2a, PG 3 variant 3a.

The availability of different carbon sources as judged by growth in their presence showed a remarkable general uniformity. Of the simple sugars and polyalcohols, glucose, galactose, maltose, lactose, cellobiose, glycerol and mannitol supported excellent growth in all cases. On the other hand, none of the strains attacked xylose, arabinose or levulose after 10 days, although later a very slight amount of growth on these sugars occurred with some strains. The growth with sucrose was generally weak, only PG 3 variant 1 showing good development. Agar and alginic acid were readily attacked except by NB 3355, which was unable to grow at all on them. Cellulose (in the form of cellophane strips) was utilized in all cases. All strains grew well on succinate and malate but were unable to use acetate, citrate and lactate, with the exception of NB 3355, which developed satisfactorily on lactate.

It is thus apparent that a definite general pattern of carbon utilization runs through the whole group. NB 3355, the authentic \( A. \ coelicolor \), is the only strain which diverges markedly from the general pattern through its inability to attack agar and alginic acid and its good growth with lactate. The three variants of PG 3 which were included gave results differing in no essential respect from those obtained with the other agar-decomposing strains.

The cultural studies on these nine strains showed once again the worthlessness of criteria such as pigmentation on different media and proteolytic activity now commonly used in the differentiation of \( Actinomyces \) species. In spite of the fact that all strains (with the possible exception of NB 3355) were undoubtedly closely related, there were in many cases marked cultural divergencies between them, which stood out in contrast with the general uniformity exhibited in the utilization of different carbon sources. Furthermore, in a number of cases there were considerable differences between cultures of a single strain grown on Czapek's agar, depending on the temperature of incubation. For these reasons it seems unnecessary to give details of the results.

The only reliable distinction between the authentic culture of \( A. \ coelicolor \) and the agar-decomposing strains would appear to be the inability of the former to attack agar and alginic acid, which can hardly be considered sufficient reason for a specific separation.

DISCUSSION AND POSSIBLE INTERPRETATIONS OF THE VARIATION PHENOMENA

Let us consider first the biochemical variation exhibited by the PG strains during the early stages of their cultivation. The 95–5 ratio of apparently non-
agar-decomposing to agar-decomposing colonies occurring irrespective of which type was used as inoculum, together with the fact that the apparently non-agar-decomposing type would produce the enzyme gelase after a considerable time lapse, indicate the common origin of both types. Conidia were always used in making transfers, so that each successive generation developed through the conidial stage. The conclusion is thus unavoidable that the process of conidium formation was in some way responsible for the observed phenomena.

The constant ratio maintained between the two biochemical types on sub-culture from either one is suggestively similar to the quantitative relationships reported by Bunting (1940) in her studies on color variations in *Serratia marcescens*, except that in the latter case the constant ratios were obtained only gradually over several transfers. It is possible that the same sort of mechanism operates in both cases, but my data are insufficient for a strict comparison.

Since agar was the sole source of carbon and energy—apart from incidental impurities—in the medium employed, it would seem reasonable to assume that the differences in colony size, pigmentation, etc., between the two types were ultimately due to a difference in the rate of gelase production. This assumption gains further support from the fact that the elimination of the difference in the rate of gelase production which resulted from repeated transfer on the same medium also eliminated the other differences.

At first it seemed that such behavior could be explained on the assumption that gelase was an adaptive enzyme in the slow agar-decomposing colonies and constitutive in the rapid agar-decomposing ones. On this hypothesis, however, one ought to find that continued cultivation in a medium devoid of agar would result in a loss of the ability to produce gelase immediately on return to an agar medium for at least a considerable proportion of the organisms. Such was not the case; several cultures which had attained a uniform rate of agar decomposition were carried through a series of transfers over a period of two months in liquid media containing glucose, galactose or glycerol as carbon sources, without the slightest diminution in the rate of gelase production being apparent on their return to agar.

The task of attempting an interpretation of the variations in colony form is equally difficult. For several reasons it does not seem possible to fit these observations into the well-known dissociative pattern of bacteria. The number of sharply distinct variant types was far greater than the number to be expected on the basis of M-S-R changes, and there were very few types which could be regarded as intermediate (i.e., SR) forms. Furthermore, there was no very obvious regularity or predictability about the changes observed—the more unstable variants gave rise at once on transfer to a wide variety of totally dissimilar types instead of to the chief dissociative form and intermediates which one normally finds in bacterial dissociation. It must be admitted, however, that the number of variable characters studied simultaneously in this case is far greater than with most bacteria.

A survey of the results leads to a few tentative conclusions as to the probable changes which may occur. Outstanding in this connection is the effect of
conidium formation. Colony types which can be transferred in the mycelial stage—either because the formation of conidia-bearing aerial hyphae is delayed, or because the aerial hyphae are devoid of conidia, or because an aerial mycelium is never formed—are likely to remain stable. This contention is supported by the behavior of types 1a, 2b1, 2b3, 2c and 4a. That their stability may be ascribed to the lack of conidium formation is shown by the results obtained when transfers of 1a were made after an aerial mycelium had developed. On the other hand, variants producing an aerial mycelium with conidia are not necessarily unstable; this is outstandingly exemplified by type 2a, which, in spite of the fact that it always produced conidia in abundance, remained the most consistently stable of all the variants.

Thus my observations would seem to indicate that it is primarily the process of conidium formation which is responsible for the great variability of the Actinomyces strains studied. That other mechanisms of variation, operative through a non-conidial phase of growth, are to be found in the Actinomyctales, has been shown beyond doubt by the work of Krassilnikov and Tausson (1938) on Proactinomyces species. It may be that the phenomena are homologous, but at present we do not have sufficient evidence to form an opinion on that point. Consequently the following discussion of possible mechanisms is limited to the conidial type of variation.

The cytological findings of Badian (1936) are of considerable interest in connection with the present work, since they provide a possible basis for explaining conidial variation. Badian claimed that the chromatin material of actinomycetes is distributed through the hyphae in the form of chromosome-like bodies which undergo autogamy just before conidium formation, with the result that each conidium contains a bivalent chromosome. When the conidium germinates, this bivalent chromosome undergoes two divisions, one of them a reduction division. At the same time, from one to three germ tubes are formed, each of which receives one of the four daughter chromosomes. The remaining chromosome or chromosomes suffer a gradual degeneration. This work has, it is true, been sharply criticized by Schaede (1939) and von Plotto (1940). However, the negative results obtained by these investigators cannot logically be advanced to disprove Badian's observations, supported as these are with excellent photomicrographs. The sole explanation offered by Schaede, that the bodies which Badian took for chromosomes were actually condensed cytoplasm, is not a convincing one. Furthermore, cytological studies by Badian on myxobacteria (1930, 1933) and on yeasts (1937) in which he used the same technique as for his actinomycete studies, have checked to a considerable extent with those of Beebe (1941) on myxobacteria and Rochlin (1933) on yeasts in which different techniques were employed. For these reasons it would seem premature to regard his observations either as figments of the imagination or as artefacts resulting from the use of faulty methods.

Badian has used his own observations to explain the phenomenon of sector formation in actinomycetes as follows:
"Zytologische Erscheinungen, wie sie oben beschrieben wurden, machen es nun wahr-scheinlich dass Sektorenbildung und Mendelsche Spaltung wirklich wesensverwandt sind. Da das Chromosom der Spore bivalent ist und zwei univalenten entspricht, die sich auto-gamiisch vereinigt haben, so muss es nicht unbedingt immer homozygotisch sein. Ist es aber heterozygotisch, so werden während der Chromatinreduktion zwei Chromosome das eine, die zwei anderen aber das andere Gen von einem Allelomorphpaar erhalten. Wachsen nun solch einer Spore drei Keimschläuche hervor, so müssen zwei von ihnen gleichartige Chromosome enthalten und darum erbgleich sein, der dritte wird aber ein im genetischen Sinne abweichendes Chromosom aufweisen. Wachsen nun die Keimschläuche aus, so wird die Zone, die aus Verzweigungen dieses dritten Fadens besteht, sich als ein Sektor mit abweichenden Eigenschaften hervorheben."

Of course this explanation covers only those cases where a single sector is formed, not examples (like that illustrated in fig. 11) of multiple sector formation. However, it is quite possible that multiple sectored colonies have developed from more than one conidium, in which case Badian’s explanation would still remain valid. Furthermore, by assuming that in many cases only

one or two germ tubes grow out from a germinating conidium, one can use Badian’s hypothesis to explain that marked heterogeneity so often characteristic of the progeny of unstable strains. Colonies developing from a single germ tube will (if we accept Badian’s explanations) always be homozygous, while at least 30 per cent of those developing from two germ tubes will be so. Thus a heterozygous condition in an Actinomyces colony would be expressed in the appearance of separate and distinct colony types if transferred through the conidial stage, but not if transferred in mycelial state. Cases of extremely stable strains producing an abundance of conidia (e.g., 2a) can also be explained on the assumption that such strains are completely homozygous. This is not to be taken as more than a mere working hypothesis, wholly dependent as it is on the outcome of future cytological work.

SOME REMARKS ON THE TAXONOMY OF ACTINOMYCES SPECIES

Although the limits of the genus Actinomyces are fairly clear, species differentiation is in a most unsatisfactory state. Because of the proven unreli-
ability of nearly all the differential characters which have been suggested or used, it has seemed to many that the attempt to erect valid species is doomed to failure. The most notable example of this pessimism is to be found in Lieske (1921) who, after years of work with these forms, rejected the possibility of a satisfactory systematic treatment.

Since Lieske's time there has been little published which would justify a more optimistic outlook. The painstaking morphological work of Ørskov (1923) laid the foundations for a better understanding of fundamental morphology in the Actinomyetales as a whole, but provided no help for species differentiation. More recently, Waksman (1940) has recognized five sub-groups in the genus *Actinomyces* on the basis of the structure of the sporulating hyphae, but this treatment provides at best only a partial solution to the problem, since in each of these sub-groups there are a number of species which must ultimately be differentiated by other means.

Conn and Conn (1941) have made what promises to be an extremely important contribution from the physiological aspect through their attempt to re-evaluate the character of chromogenesis. They have stressed the fact (which, as they say, has long been an open secret among students of the group) that many actinomyete pigments act as hydrogen ion indicators, and they have pointed out that the nature of the pigment, rather than the color produced, is the important character. The case of the litmus actinomyetes affords a striking demonstration of their contention. Actually the behavior of the pigment in this group was clearly described by Beijerinck in 1913; nevertheless, even this outstanding example of an indicator pigment has been overlooked or misunderstood. In the fifth edition of Bergey's Manual no mention is made of it in the description of *A. coelicolor,* although hints are provided for the observant taxonomist in the key:

1. Pigment blue, not always definite.
   b. Soluble red pigment, turning blue on synthetic (sic) agar.

52. *Actinomyces coelicolor.*

One point of some importance has gone unmentioned by the Connss; namely, the possibility that more than one pigment may be produced by an organism. In the strains which have been the object of the present study, a pale yellow pigment is often produced.² It is not observable in cultures producing an abundance of the litmus pigment, but when the latter is present in only slight amounts the yellow pigment often causes cultures to appear green. This shows that in order to use the pigmentation as a differential character it will be necessary not merely to record the pH of the medium but to make an extraction of the culture, a separation of the pigment components and a study of the behavior of each one alone. Such studies must be carried out with a variety of media, since on certain substrates the production of the characteristic pigment may be slight or even absent. It remains to be seen in how far there

² This was also noted by Beijerinck (1913) in the strains studied by him.
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occurs a permanent loss of the ability to produce pigments of special types; the Conns consider this character to be stable, but there are some indications from my work that unpigmented variants (e.g., 2b3 and 4a) can be derived from litmus actinomycetes.

Finally, I should like to draw attention to the possibility that the utilization of different carbon sources may prove a desirable taxonomic criterion. It is true that attempts to use a single character of this type (e.g., the ability to decompose cellulose) as was done by Krainsky (1914) have not been successful, but the general pattern of carbon utilization in actinomycetes has never been subjected to a systematic investigation. The remarkable uniformity with respect to carbon sources in the strains I have studied suggests that in future this may be a profitable approach. Since the majority of soil actinomycetes will grow in a medium with mineral nitrogen alone, the experimental technique adopted by den Dooren de Jong (1928), which makes easy the simultaneous testing of a large number of strains on a wide variety of carbon sources, could in most cases be employed to advantage.

In conclusion, I should like to express my thanks to Dr. C. B. van Niel and to Dr. A. T. Henrici for their valued criticism and advice.

SUMMARY

Attention is drawn to the existence, previously unrecorded, of agar-decomposing organisms among the Actinomycetales. Several strains have been studied and shown to belong to the Actinomyces coelicolor species-group.

On first isolation several of these strains gave rise to colonies which varied in their ability to attack agar, but on continued cultivation the differences in this respect disappeared. During the subsequent work marked variations in colony form, conidium formation, pigmentation, and other characters were noted. Some of these variants were stable, whereas others continuously gave rise to new types.

An analysis and interpretation of the possible mechanisms of variation in these organisms has been attempted.

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