ON THE PROPOSAL FOR A NEOTYPE OF
STREPTOMYCES ALBUS: IDENTITY OF THE
PRESENT DESCRIPTION WITH THE ORIGINAL ONES BY
GASPERINI AND ROSSI DORIA

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SUMMARY. Strains already studied by Pridham and Lyons for the proposal of a *Streptomyces albus* neotype have been re-examined. We maintain that the descriptions of strains conform exactly with those published by Gasperini and Rossi Doria. Therefore we reject objections brought up in this connection by Pridham and Lyons. However we accept strain ATCC 3004 as the neotype of *S. albus*.

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INTRODUCTION AND PURPOSE OF THE WORK

It seemed proper in our opinion to resume a study of *Streptomyces albus*, notwithstanding the recent and most excellent researches undertaken by Pridham and Lyons (1961, 1962) and by Hütter (1961). In fact we do not share their interpretation of the original papers by Gasperini (1890) and Rossi Doria (1891) who after all gave the first description of the species. In this paper, after researches and comparison of the different strains studied by Pridham and by Hütter, we will consider in detail the points of disagreement.

MATERIALS AND METHODS

a) - Strains studied

The examined strains and their origin are as follows:

1. 3004-a: received from Pridham as *Streptomyces albus* NRRL B-2208 (IMRU 3004).
2. 3004-b: received from ATCC as *Streptomyces albus* ATCC 3004.

3. 618: received from ATCC as Streptomyces albus ATCC 618.
4. 82x: our isolation (IPV) from beehive (Strain No. 1 of Stockholm Cooperative Experiment).
5. 298x: our isolation (IPV) from diseased bees.

Concerning the history of the first three strains and their possible identity with Berestnev and Krainsky isolations see Lyons and Pridham (1962).

b) - Methods

Cultural studies were carried out using the following media: potato agar, Czapek solution and agar, casein Czapek agar, glycerol asparagine agar, Ca malate agar, nutrient agar, international peptone agar, peptone iron agar, starch agar, peptone broth, potato and carrot slants, tyrosine agar, skim milk, gelatine, butter, cheese, cooked eggs, fruits.* Cultural characters were examined in tubes and petri dishes.

The morphology of the sporophores was determined by direct microscopic examination of petri dish cultures using the above listed media.

Observations have been carried out with transmitted as well as with incident light (Leitz Ultropak).

Spore morphology was determined by examination of impression mounts with the electron microscope.

The following biochemical characters were examined:

Nitrate reduction: on organic and synthetic media.

Gelatine liquefaction: in test tubes (12 percent gelatine) and in petri dishes (Czapek agar + 0.4 percent gelatine) SAB Manual (1957).

Hydrogen sulfide production: lead acetate strip test on peptone iron agar + 0.1 percent yeast extract (Difco).

Caseinase activity: Czapek agar + 0.4 percent skim milk (Difco), following the techniques described by Tendler and Burkholder (1961).

Diastatic activity.

Ability to decompose tyrosine and xanthine.

Ability to utilize different carbon sources.

* Natural substrata have been chosen because of their use by Gasperini and Rossi Doria.

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As a general procedure the techniques listed in "Methods for use in cooperative studies on criteria for description of the streptomycetes" June 1958 (unpublished), and by Pridham and Lyons have been followed.

c) - Results

Our results conform to those obtained by Pridham and Lyons. Strain 3004-a, received from Pridham as a lyophilized culture, appeared less typical than strain 3004-b.

As already stressed by Lyons and Pridham (1962) we observed a variability of the ability to reduce nitrates according to the media used.

Production of hydrogen sulfide: as already observed by Pridham and Lyons none of the strains gave positive reaction on peptone iron agar (Tresner-Danda test). However, we would like to stress that according to the observations by Turri and Silvestri (1960) and by Küster and Williams (1964) the bluish-black coloration of substrata is only indicative of melanin pigments production. In fact, using the "lead acetate test" technique on the same medium, good H₂S production (blackening of the strips) has been obtained without any pigmentation of the medium.

DISCUSSION OF THE RESULTS AND
CRITIC-HISTORIC SURVEYa) - Description of Streptomyces albus according to Gasperini and Rossi Doria.

For a comparison of our results with the studies by Gasperini and Rossi Doria, we think it would be useful to give a summary of the descriptions by the Italian authors, since their work seems to be little known. Rossi Doria does not give an extensive description of Streptotrix alba but refers completely to Gasperini. This may suggest an exchange of strains between the two authors. At present we are not taking into consideration the nomenclature used by the authors and already criticized by one of us (Baldacci 1939). In Tables I and II we summarize the characters of Streptomyces albus according to Gasperini and Rossi Doria.

Table I. Summarized description of Streptothrix foersteri according to Gasperini - 1890.

Media	Vegetative mycelium	Aerial mycelium	Morphology	Biochemical activity
Nutrient gelatine at 15°-18°C	colourless	whitish	Long, isolated serpentine filaments and spirals	Gelatine liquefaction
Agar at 37°C	colourless, rich growth	white		
Blood serum	good growth	good sporulation		
Black bread	very slow growth at acid reaction			
Cooked potato	good growth			
Egg coagulate albumine	slow growth			
Peptone broth	good, quick growth	good sporulation		
Mineral solution	growth on the surface	good sporulation		
Distilled water	no growth			

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Table II. Summarized description of Streptothrix alba
according to Rossi Doria.

General characteristics:	good growth on vegetable and animal substrata; white aerial mycelium with filaments forming concentric rings. No anaerobic growth. Mouldy smell. Air isolate.
Gelatine:	no soluble colour
Milk:	before coagulation milk becomes transparent. Alkaline reaction.
Butter:	no growth.
Cheese:	no growth.
Cooked egg:	good growth with white aerial mycelium on yolk.
Marrow:	quick growth.
Carrot:	quick growth.
Cereal and legume meal:	quick growth.
Melon:	good growth.
Fruits and compact parenchyma:	no growth.
Meat:	good growth.

b) - New description by Pridham and Lyons and agreement with our results

As already mentiond, Pridham and Lyons (1961, 1962) made a proposal to designate strain ATCC 3004 as the neo-type of Streptomyces albus. The results of these authors conform to our own. From our survey ensues a definite identity of the description by Gasperini and Rossi Doria with the one by Pridham and Lyons. On the contrary, the American authors maintain that they have noted some disagreements. These however, in our opinion, are insignificant and we are now going to examine them in detail.* The controversial points are:

1. Distribution in nature: the question as to the greater or lesser degree of diffusion in nature of Streptomyces albus is in our opinion a purely formal one. Up to 1891, that is including the work done by Rossi Doria, the streptomycetes which had been isolated and studied, including S. albus, were not more than eight (Baldacci 1958). Six of them were pathogenic isolates and two had been isolated from the ambient. Among the last ones S. albus was the most frequently isolated; however, this does not mean that it is also the most widespread in nature.
2. Formation of concentric rings in aerial mycelium: In our opinion this is not an exclusive characteristic of Streptomyces griseus. The formation of rings may be assumed to be related with methods of cultivation (variable temperature, light, etc.) very different from present conditions in our laboratories. Besides we may add that just one of the species (Actinomyces annulatus) of Krasil'nikov's (1959) Albus possesses as a distinctive character ring -formation in aerial mycelium.
3. Structure of sporophores: There are many passages in Gasperini's paper clearly indicating the sporophores structure and in particular the presence of spirals. According to the author at 150 enlargements at the edge of the colony long isolated filaments can be observed. The

* These points have already been discussed personally with Dr. Pridham.

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filaments "s'éloignent en décrivant des courbes irrégulières, saillantes, et le plus souvent serpentrantes" (Fig. 6, pl. VI). At 450 enlargements the filaments appear more compact "tantôt droits, tantôt recourbés ou en forme d'hélice" (Fig. 7, pl. VI). In our view we would rule out the possibility that this description refers only to vegetative mycelium. Fig. 6 of Table VI does not seem to us to leave any doubt on this point. Fig. 7 of the same table also gives the idea of an aerial mycelium in its formation process. It is well known that not only vegetative mycelium can be found at the edges of the colonies but fairly advanced development of aerial mycelium too may be observed. Everything depends on how the term "edge" should be interpreted. At the above-mentioned enlargements the structure of the colony under observation is still fairly complete. Figs. 5 and 6 of Table VII, to which Pridham and Lyons refer have been taken at greater enlargements (1500x). Under these conditions and with the techniques available at the time it seems right to assume that the structure of the sporophores would have been mostly broken up. However Fig. 1 of the same table taken at 1450 enlargements clearly shows a number of spirals. Therefore we may come to the conclusion that the description by Gasperini undoubtedly refers to sporophores from flexuous to spiral.

4. Colour: both Gasperini and Rossi Doria make definite statements about the presence of a white colour of sporulated aerial mycelium. According to Gasperini the colonies already forming spores between the sixth and eighth day are white ("tres blanche") and persist for a long time. Dealing with another species Rossi Doria speaks of a pure white powder ("polvere blanchissima") exactly like that of Streptotrix alba cultures. On the other hand the name alba (= white) was chosen by Rossi Doria specifically in relation to the aerial mycelium colour. We feel that it would not conform to the text to assume that the substrata used by the Italian authors does not lead to recognition of good sporulation since there are clear indications of both good and poor sporulation. In fact Gasperini carefully points out (p. 458) cases where no spores are formed (gélose abondamment glycérinée).

Furthermore, during our own researches we could observe very abundant growth and sporulation on potato and carrot slants, sometimes better than those obtained on ordinary media.

In conclusion we think we can state that the descriptions by Gasperini and Rossi Doria and the binomials they proposed should be included as S. albus synonyms (see Baldacci 1939) and should in no way refer to Streptomyces griseus. According to our opinion the objections brought out by Pridham and Lyons are lacking in a legitimate interpretation basis.

SUBSEQUENT REFERENCES AND DESCRIPTIONS OF S. ALBUS

A revision of new references and descriptions of S. albus, according to Rossi Doria, has been already carried out by one of us (Baldacci 1939) and later by Hütter (1961). However we think it useful to take into consideration some of the descriptions that stand, aside from the original one by the Italian authors.

1) - Krainsky (1914)

No morphological characters are given by Krainsky. Vegetative mycelium is colourless and aerial mycelium white. However on gelatine broth and glucose agar a gray colour of the aerial mycelium is reported.

2) - Drechsler (1919)

According to Drechsler's description the organism shows the following characters:

- a. On potato agar aerial mycelium white, changing rapidly to a yellowish gray.
- b. Substratum stained a faint greenish yellow in old cultures.
- c. Fertile hyphae show no indication of a spiral condition.
- d. Short cylindrical spores.

All these characters lead one legitimately to believe that his strain is closer to the species S. albidoflavus than to S. griseus. Moreover Drechsler puts a question mark after this last synonym.

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3) - Waksman (1919)

Waksman himself states in advance: "this organism resembles in certain respects the one described by Krainsky and before by others, although it is doubtful whether it is the same organism as the one that Krainsky described." We want to stress some items in Waksman's description which do not agree with previous and subsequent findings:

- a. Ca malate agar: gray aerial mycelium (Krainsky: white aerial mycelium).
- b. Glucose agar: pale mouse gray aerial mycelium (compare statement by Krainsky).
- c. Starch agar: no aerial mycelium.
- d. Nutrient agar: few white patches of aerial mycelium.
- e. Potato plug: thin white aerial mycelium, only covering edge of growth; colour of plug purplish with age.
- f. Carrot plug: very thin, white aerial mycelium.
- g. Diastatic activity: broad (10-15 mm) enzymatic zone.
- h. Gelatine: brown soluble pigment; medium liquefaction.
- i. Milk: brownish surface ring of growth.
- j. Morphology: no spirals on synthetic agar, straight branched mycelium. A few short, closed spirals, are found on the glycerin-synthetic agar.

Furthermore according to Pridham and Lyons (1962) "strain IMRU 3005, designated as the type culture of S. albus on page 172 of the Waksman volume 2 (1962), has characteristics that conform to those of S. griseus, based on our study of a culture of the strain obtained directly from Waksman."

In conclusion it should be stressed that a different concept of S. albus was put forward just after Gasperini's and Rossi Doria's studies. However, it is difficult to assume that it is a question of one concept only. The literature examination leads to the following statements:

1. That Drechsler did not grasp the difference between S. albus and S. albidoflavus.
2. That Krainsky did not distinguish the present species S. griseus from other species falling, in our opinion, within the series Diastaticus.
3. That Waksman made his amendment on a strain possessing characteristics rather similar to those described by Krainsky.

4. That neither Krainsky nor Waksman took into consideration the descriptions given by the Italian authors.

CONCLUSIONS

From the above-reported descriptions and comments we feel that strong enough demonstration has been brought up to show that the descriptions by Krainsky, Drechsler, and Waksman definitely differ from the originals.

Therefore in our view the name Actinomyces albus Krainsky 1914 should be transferred to the synonyma dubia, contrary to the previous statement by Baldacci (1939). The same should be done with the already dubitative nomenclature used by Drechsler and with Actinomyces albus (Krainsky) Waksman et Curtis 1919. For the last nomenclature reservations had already been made (Baldacci 1939).

As general conclusions we believe:

1. That the description of the neotype by Pridham and Lyons for S. albus diagnosis should be accepted.
2. That their new diagnosis conforms completely with the descriptions given by Gasperini and Rossi Doria, as we have demonstrated.
3. That the objections brought up by Pridham and Lyons against this identity have no interpretational legitimacy.
4. That the Krainsky, Drechsler, and Waksman diagnoses conform neither to the original nor to the present description.

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