COOPERATIVE DESCRIPTION OF TYPE CULTURES
OF STREPTOMYCES.
I. THE INTERNATIONAL STREPTOMYCES PROJECT¹

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Adequate taxonomy of microorganisms is difficult because of the relative ease with which living entities vary. Even the simplest of living cells has a complexity exceeding by orders of magnitude that of an inanimate machine. The taxonomist accepts variation as part of the natural order, but since his identification of microbes will depend on the characters of the species, he seeks to identify the most stable characters that he can recognize; knowledge of these is the first step in taxonomic investigations (3, 9).

The identification of Streptomyces species involves many difficulties. The realization in the past 20 years that the genus Streptomyces was the touchstone in the search for new antibiotics, led to a search for new isolates that would produce such therapeutic agents. This intense search increased knowledge of the Streptomycetes for many new forms were, in consequence, isolated and carefully studied. Many strains of Streptomyces were validly published as new species until now there are about 600 named species. It may well be that many such species should be regarded as subspecies or even as infrasubspecific forms.

Noteworthy in an evaluation of species descriptions is the fact that characters that had been used for species descriptions before 1940 were usually based on the characteristics of the organism when grown on unstandardized media. Furthermore, many of the diagnostic criteria that were used were so vague as to be almost useless as an aid in species

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determination; other criteria were too variable to be of value. Often, the published descriptions were quite inadequate for identification.

Because of this inadequacy, the investigators tried to obtain authentic type cultures for comparison and adequate description to determine whether an isolate was similar to a previously described species or was a new species. However, authentic type or neotype cultures of many published species were not available or did not even exist, and neotypes for missing type cultures had not been proposed. The availability of some apparent "type" cultures did not always help and at times confused the situation even more. There were instances where two different strains of the same species were called type cultures and other instances in which the so-called type cultures no longer resembled the original descriptions.

On petition of a group of taxonomists interested in the actinomycetes, the American Society for Microbiology constituted a Committee on the Taxonomy of Actinomycetes in 1958. This later became a subcommittee of the Committee on Taxonomy.

The subcommittee stimulated research in its area by first collecting funds and allocating research grants. It organized symposia and sponsored round table discussions. However, it became evident that an attempt should be made to secure more accuracy, precision and uniformity in methods of judging taxonomic characters. Workshops were held in an effort to compare the judging and evaluation of cultures. Cooperative studies were undertaken by both the Committee of the American Society for Microbiology and the Subcommittee on the Actinomycetales of the International Committee on Nomenclature of Bacteria.

The ASM Subcommittee proposed:

1. A test to determine whether cultures of species of Streptomyces known by a code number could be identified with the aid alone of the descriptions and keys that were available (unpublished material);
2. A test of the reproducibility of results in tests using current descriptive criteria (2, 4, 5, 6).
3. An international investigation of color judgement by different systems of evaluation (7).

The data were secured and the subcommittee was prepared for its next step, the collection of authentic type cultures and a description of these cultures by the best available methods. Dr. Elwood B. Shirling of Ohio Wesleyan University agreed to be Director of an International Streptomyces Project. A research grant from the National Science Foundation allowed the further development of this program (GB-4367).

The basic philosophy of the program was that, to be of value, a description of any species must be reproducible by competent investigators anywhere in the world. Furthermore, it should be based on an authentic type culture if available. When such types were not available neotype cultures should be proposed which reasonably corresponded to original type descriptions. All type cultures should be deposited in collections so as to be freely available to investigators.

There were also limits to the extent of the studies. For example, there was to be no evaluation of the legitimacy or validity of a described species, nor would attempts be made to unite or divide species in these studies. Any such critical evaluations were to be left to individual investigators for study as they saw fit. Furthermore, the project would not include study of the specific problems of nomenclature so that generally the names of species would be those under which the cultures were received. This position was important for there was a long-standing international lack of consensus concerning the use of the generic names Actinomyces and Streptomyces. Only species of the genus Streptomyces (also called Actinomyces) and the closely related genus, Streptoverticillium, were included in the study.

The description of the type strain was not to include any attempt to delimit the species. Delimitation of the species would require a study of many strains and was beyond the scope of this program. In addition, the description of a type strain included only the characteristics considered minimal. However, investigators were asked to note additional characters which they believed to be reliable enough for distinguishing species.

The program could be executed only through international cooperation. For this purpose, the project was made cooperative between the Subcommittee on Taxonomy of the Actinomycetes of the ASM and of the International Committee on Nomenclature of Bacteria of the IAMS. Cooperating investigators were divided into groups of three. The members

of each group were so chosen that, whenever possible, no two members were from the same country. This would mean that the variation in technique among three investigators who examined each type culture must not materially influence the description. The final agreement on a description of characters of the species would tend to hold true despite a large amount of variation in technique and conditions. Some variation could not be avoided despite all attempts to standardize methods of characterizing the organisms.

The response of our invitations to potential cooperators was gratifying. Thirty-three individuals participated in studies of the first hundred cultures.

The second concept, that of the use of authentic type cultures was sometimes difficult to execute since such cultures were hard to locate. All available described species of Actinomyces, Streptomyces, and Streptovercillium were compiled both from the literature and from lists that had been gathered by some of the cooperators. This tentative list was sent to all cooperators for names of additional species to be included in a master list. From this compendium of names, requests for type cultures were sent first to the author of the species name and then to culture collections where the type might have been deposited. A list of the remaining cultures with unidentified type strains was prepared and sent to streptomycete taxonomists who were asked to help find the type cultures. This search is still underway in attempt to find as many type strains as possible.

The culture accumulation was accompanied by a compilation of the original culture descriptions. Not all cultures could be obtained, some because the type or neotype is no longer extant or could not be located. Other type cultures could not be obtained (even when in culture collections) because their distribution was denied until patent rights had been determined. Finally, some cultures of species were withheld in various countries because of the individual policy of the organization that discovered and was using them. The accessioned cultures were compared to the original descriptions before being lyophilized for distribution to the cooperators.

To aid in obtaining as much uniformity as possible in the characterization of the species of Streptomyces, a standardization in the interpretation of their characteristics and in their descriptions was necessary. A number of aids were developed. A manual of methods was compiled from results of previous investigations in this field (8). This preliminary

draft was submitted to the investigators for criticism and suggestions. Media that contained crude organic plant or animal materials were a serious problem because of the variability of the product in different countries. Through the kindness of Mr. C. W. Christensen of the Difco Laboratories, such media were prepared by them under standard procedures for the entire project.

Another area of uncertainty had to do with color. *Streptomyces* produce many pigments. Since color is one of the easiest characteristics to observe, it has always been used in descriptions and has often been the prime distinguishing characteristic. It even forms the basis of separation of large groups of species in some classifications. There has been a tendency to describe colors of *Streptomyces* in very narrow terms, i. e. dull, yellowish brown, greyish, greenish blue. Culture color, however, can vary because of slight differences in media, environment, handling, and age of culture. Furthermore, sensitivity to color differs widely. Even though color standards are used the descriptive terms used vary from individual to individual. Yet, color is such a readily observed characteristic that it cannot be disregarded. Two alternatives were apparent, either the color must be described in exact physical terms of wavelengths and intensity, or a broad grouping of colors that allow for a wide variation inside any color group must be employed. The second of these alternatives was chosen for the study since a study reported at the 1964 Congress of Botany showed it to be the most generally useful system (7). Color wheels were made according to the scheme of Tresner and Backus (10). Each wheel contained a wide range of color gradations that was included in the color group. All gradations of color within the limits of the wheel were included in that color name. This scheme allowed for a standardization of names on a broad color basis so that small variations were not considered. When a color of a culture resembled those in more than one wheel both wheel colors were given.

All type cultures were coded when received and sent unnamed to the collaborators. Only after the results of the characterizations of the cultures by the investigation had been completed were the species names distributed. These descriptions of the organisms were to have been returned by investigators to the project office within three months of the receipt of the cultures; as might have been expected, they rarely met that deadline. Yet, the cooperation was generally good and only one investigator was not able to complete his study.

The descriptions were compiled and then evaluated by a committee. When the three descriptions of an organism agreed or were quite consistent there was no problem. If the results for any character from all three investigators were different, that character was considered unsuitable for the species description. Agreement between two investigators but not the third posed problems; differences were usually settled by submitting the culture to a fourth investigator. The only characteristic that was not always examined by three cooperators was the nature of the spore surface because not all laboratories had access to an electron microscope; but in all cases electron micrographs were submitted by at least two investigators and the results were always quite consistent.

The question of custodianship of the type cultures also was of some concern. Because of the practical importance of the Streptomyces the difficulties in the movement of cultures across political boundaries and thus their availability to investigators had to be faced. The Centraalbureau Voor Schimmelcultures, Baarn, Holland (The Netherlands) was designated to receive cultures of type strains and neotype strains as proposed by the International Streptomyces Project with additional centers for distribution in the United States of America, the Soviet Union, and Japan.

The International Streptomyces Project still is a long way from its goal. It has recorded about 600 species but only 400 type cultures or potential neotype cultures have been secured; of these 300 putative species have been investigated. The acquisition of type cultures has slowed to a trickle and it is doubtful whether many more will be located. The next important step in the program is the proposal of neotypes for cultures for which type cultures no longer exist. This proposal may be by individual investigators and the cultures then incorporated in the project or three investigators could describe them directly and propose them with joint authorship.

What is to be done with species names for which no cultures can be found? The majority would propose them for rejection but a minority would rather retain them on the chance that a culture answering to that description would be found. A good many of these species have been described in such general terms that a real fit between the description of a species and a culture would have very little meaning. Unidentifiable species are not being included in the study.

The descriptions of the first hundred type species have now been compiled and will be published in the second paper of this series. These descriptions are in no sense final; they will be emended as new techniques of differentiation are developed. Other criteria should be studied for their value in taxonomy and should eventually be added to those recommended. Recently, for example, a group of three individuals competent in serology has been formed in the I. S. P. The members will examine the serological properties of the type cultures to ascertain their value in species diagnosis. Similarly, we look forward to the joint study of the use of other techniques that are now being described in the literature—such as protein profiles, infra-red absorption characteristic, base ratios in DNA, inhibition by antibiotics and many others.

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