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RESULTS OF A COMPARATIVE
STUDY OF CRITERIA USED IN THE
CLASSIFICATION OF THE ACTINOMYCETES

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SUMMARY: The results of a comparison of criteria used in characterization of the Actinomycetes under the auspices of the International Subcommittee are reported. Although this first co-operative work carried out on a large scale showed some deficiencies which were caused by the selection of the media and methods and by the type of description, some interesting evidence concerning the value of the used criteria for the classification of Streptomycetes were obtained.

The morphology of sporophores and the shape of spores are significant and constant characteristics so that they can be used as taxonomically useful criteria.

Likewise, the melanin reaction is an unequivocal characteristic and can be applied in a classification. The determination and description of the colours of the aerial as well as of the vegetative mycelium are much more complicated. In principle these results confirm those obtained from a co-operative experiment which was carried out in the USA a short time before (Gottlieb, 1951). In this study organized by the Subcommittee on Actinomycetes of the Committee on Taxonomy of the Society of American Bacteriologists workers at 11 different laboratories examined 5 different strains by means of 12 morphological as well as physiological criteria

under strictly defined methods.

Further studies are necessary to find the most useful criteria and to improve the methods which are suitable for the classification of Streptomycetes. Further co-operative and comparative work could be the best way to solve these taxonomical problems.

During the course of the Round Table Conference on Streptomycetes in Stockholm in August 1958, the participants formulated a plan for a cooperative experiment which should establish the reproducibility of the stable properties used in the characterization of Streptomycetes. In accordance with this plan (Küster, 1959) an international co-operative work has been carried out. Thirty two individuals participated in this experiment. In twenty nine laboratories 25 strains from Prof. Baldacci's collection were examined, and three other laboratories used 10 strains only. The experiments were carried out three times at definite time intervals; only one laboratory attempted them once.

The main tasks of the co-operative study were first of all to decide whether a characteristic was sufficiently constant and reproducible to be used for classification and secondly to standardize the type of description as much as possible so that the individual observations could become comparable. Both questions have not been solved completely but the results may be sufficiently interesting to justify publication.

The following criteria were employed and examined:

1) Morphology of sporophores: The different forms were described according to the nomenclature of T.G. Pridham et al. (1958) with only a few modifications as proposed by Baldacci. The morphology of sporophores is a significant and constant characteristic. Nineteen of the 25 test strains were determined unequivocally and in the same way. The differentiation between spiral and non-spiral was quite easy. Those between *Flexibilis* and *Retinaculum* and between *Rectus* and *Flexibilis* respectively seemed to be not so simple. Possibly the scheme used for the determination of the morphology of sporophores as one of the main characteristics with taxonomical value could be simplified for future studies which would lead to a division into groups, series, and the like.

2) Form of spores: The examination of the spores by means of an electron microscope was carried out in 22 lab-

oratories. The results were clear and definite. The differentiation between smooth and spinous spore surfaces was simple. Dissenting observations were very rare. This shows that the shape of spores is also a significant and constant characteristic so that it can be used as a taxonomically useful criterion.

3) Melanin-reaction: A 100% or nearly 100%-decision for positive or negative was very frequent. An uncertainty occurred very seldom. In the most extreme case the deviation was not more than 16%. The negative melanin reaction was distinctly easier to determine than a positive reaction which always also showed deviations. Consequently the melanin reactions seems to be an unequivocal characteristic which can be applied in a classification because of its advantage of showing only two alternatives of the determination (+ or -). Intermediates of doubtful determination are rare. In the opinion of some workers (Tresner and Dange, 1958, Hütter, 1961) these intermediates can be eliminated by replacing the melanin reaction by the H_2S reaction. This reaction gives results which are almost always in accord with those of the melanin reaction and can be described more clearly and more unequivocally as positive or negative.

4) Colour of the aerial mycelium: A clear determination of the colour of the aerial mycelium is very difficult so that one dominant colour could be determined with only 17 strains. The colour of the aerial mycelium is very rarely so clear that it can be classified into one of the 9 suggested colours, white, yellow, orange, pink, red, blue, green, gray, brown. Mostly various intermediates and shades occurred which were allocated to two or more basic colours. Furthermore, the determination of colour is rather a personal matter, more or less subjective. Each observer has not only a different personal perception of colours, but he may also determine the same culture differently at different times, particularly when dealing with cultures showing coloured intermediates which are termed white one day and gray another. The kind of light is also important, bright or gloomy daylight or artificial light influencing the determination of colour. The difficulty in determining the colour is more a matter of describing and interpreting than of the variability of the colour. In my opinion the variability of this characteristic is not the main reason for this complicated situation. The subjective determination is too variable, and an exact stan-

standardized description was not possible under the conditions of this experiment. The use of standard colour plates with code numbers may partly eliminate these difficulties because they also comprehend the intermediates, but the subjective manner of observing and describing is not completely excluded. For the division into larger taxonomical units such as groups, series, and the like it is necessary to fix which colour numbers, i. e. which intermediates, should be attached to one basic colour. The best solution might be a method by which the colour would be determined objectively, e. g. by spectrophotometric measurements. N. Paulin (1960) started some experiments using this technique. Although the results are not yet sufficiently satisfying they may be the basis for further studies in this direction.

5) Colour of the vegetative mycelium: All the above mentioned facts which make a determination of the colour of the aerial mycelium so difficult occur to a much greater extent in determining the colour of the vegetative mycelium. Here, a dominant colour could be chosen with only 9 strains. In some cases up to 9-10 different colours were observed. It may be interesting to note that some of these strains also showed the greatest variation in the colour of the aerial mycelium. Even more than for the determination of the morphology which is constantly and equally distinct on all substrates, the selection of suitable media is important for the determination of the colour. Because of different requirements of the individual species and strains it is difficult to find one optimal medium which produces a strong aerial mycelium with distinct colour in all cases and makes an observation of the colour of the reverse side of the vegetative mycelium also possible. But for the determination of a taxonomic characteristic it is absolutely necessary to apply the same medium always even if it is not optimal for all strains. This does not exclude the additional description of the growth on a special medium, in particular if it is quite different from that on the standard medium.

One of the most important tasks of the co-operative work was to decide whether a characteristic was sufficiently constant to be used for classification. In our case all the observations were carried out three times at definite time intervals of two months. It might be interesting to state which observation produced the greatest number of dissenting results. An observation different from the dominant deter-

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mination of one strain by one worker is termed dissent. In this way the first observation can also be a deviation. The other alternative of adopting the first observation always as the basic one has not been taken into consideration. Table 1 indicates the sum of deviations in all strains with regard to each characteristic.

Table 1. Aberrations of the dominant observation.

Characteristic	Observation			
	1st	2nd	3rd	Totals
Morphology of sporophores	48	38	59	145
Forms of spores	--	4	1	5
Colour of aerial mycelium	80	49	60	189
Colour of the vegetative myclium	96	68	64	228
Totals	224	159	184	567

This confirms the above explained variations in the observation of the characteristics, inter alia, that the determination of the colour of the vegetative mycelium shows the greatest degree of variation. The greatest number of deviations with regard to colour determination is recorded in the first observation, but this need not be caused by a variation of the colours within the subsequent observations. It might also be suggested that the observer got accustomed to the proposed scheme during the repeated observations.

It may always be difficult to divide the natural appearances which occur as various intermediates into a hard and fast scheme. Only by many individual observations can a large sample of figures be obtained which can be evaluated statistically so that the dominant characteristic can be seen more or less distinctly. In this way a 100%-decision was given if one worker unequivocally observed only one form or colour respectively within one strain. In the other cases the critical examination was not 100%, but only 50, 33, 25, 20% etc. The following statement may also be interesting to demonstrate whether the so-called 100%-decision originated always

from the same worker. Most of the workers could classify their observations concerning the morphology of sporophores into the scheme more easily than their determination of the colours. Some workers gave a 100%-decision very frequently with each characteristic, but this must not mean that they are less critical than the others but only that they noted the dominant tendency as determination on the filing cards.

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REFERENCES

- Gottlieb, Dr. 1961. An evaluation of criteria and procedures used in the description and characterization of the Streptomyces. Appl. Microbiol. 9: 55-65.
- Hütter, R. 1961. Zur Systematik der Aktinomycceten. V. Die Art Strept. albus (Rossi-Doria emend. Krainsky) Waksman et Henrici 1943. Arch. Microbiol. 38: 367-383.
- Küster, E. 1959. Outline of a comparative study of criteria used in characterization of the Actinomyccetes. Internatl. Bull. Bact. Nomen. and Taxon. 9: 97-104.
- Paulin, N. 1960. La valutazione oggettiva del colore nelle colonie di Attinomiceti. Giorn. Microbiol. 8: 91-114.
- Pridham, T.G., C.W. Hesseltine and R.G. Benedict. 1958. A guide for the classification of Streptomyccetes according to selected groups. Appl. Microbiol. 6: 52-79.
- Tresner, H.D. and F. Danga. 1958. Hydrogen sulfide production by Streptomyccetes as a criterion for species determination. Jour. Bact. 76: 239-244.