COMMENTS CONCERNING THE TAXONOMY OF THE
GENERA MICROCOCCUS AND STAPHYLOCOCCUS¹

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The Editorial Board of this Bulletin (1954) has summarized concisely the current status of the generic names Micrococcus and Staphylococcus, and has asked for comments and suggestions.

The first question raised is whether Micrococcus luteus (Schroeter) Cohn is a recognizable species. Breed (1952) has reviewed the literature and indicates that this species should be defined as non-liquefying, non-nitrate reducing, yellow-pigmented coccus capable of utilizing ammoniacal nitrogen as a sole source of nitrogen. He also suggests that ATCC culture 398 would make a satisfactory type culture. Gibson (1953) rejects the validity of this species because the suggested type strain does not grow in an ammonium tartrate-salts medium.

We have studied recently ATCC culture 398, in addition to a collection of named strains of micrococci, kindly supplied by Dr. Breed. In confirmation with Dr. Gibson's claim, strain 398 failed to grow in Cohn's ammonium tartrate medium or on Hucker's ammonium phosphate-glucose medium. Nine of the strains in the collection were reported to have grown in such media when first isolated, but at the present time only 2 of these 9 strains retained this characteristic. Further investigation revealed that the cultures failed to grow because of their inability to utilize tartrate or glucose as an energy source. Our present knowledge of the nutrition of these organisms also indicates that these tests are dependent upon carrying over the required vitamins in the inoculum. Therefore, we consider such tests for ammonia utilization inapplicable to this group of organisms.

¹Journal paper No. 113, American Meat Institute Foundation.

In spite of these findings, we do not feel that the species Micrococcus luteus should be invalidated or strain 398 rejected as the type culture. We feel that the species should be defined without reference to utilizable sources of nitrogen. Strain 398 is at the present time an obligate aerobic organism that obtains its energy from oxidation of the nitrogenous components of complex media. Although Hucker (1928) indicates that Micrococcus luteus strains produce acid from glucose and mannitol, these tests were performed by incubating agar slope cultures for as long as 30 days. He considered these "fermentation tests" to be of little value because the results were so frequently altered by changes in the nature of the inoculum, the composition of the basal medium, and the conditions of incubation (Hucker, 1924). Our present knowledge of microbial physiology and nutrition may enable us to eliminate these variations by limiting the term "fermentation" to the anaerobic utilization of a substrate as an energy source in a standardized basal medium.

We are also convinced that the coagulase-positive staphylococci represent one of the most homogeneous species of bacteria to be found in any genus (Evans, 1948; Evans and Niven, 1950). Our contention is supported by the studies of numerous earlier investigators and by the more recent studies of Shaw, Stitt, and Cowan (1951). We feel that this should be recognized as the type species of the genus Staphylococcus. There seems to be a reasonable doubt as to whether the correct specific epithet should be aureus or pyogenes. In view of the fact that orange strains frequently give off white variants and these variants, as well as naturally-occurring white strains, are otherwise indistinguishable from orange strains, we feel that the specific epithet pyogenes should be accepted.

If we recognize the validity of these two species, the next major problem is to decide whether they should be placed in the same genus or in separate genera. If they are to be placed in separate genera, we are then faced with the problem of where to draw the line of separation between the two genera.

We feel that such a separation is both desirable and possible, and therefore it is proposed that the separation be based primarily on the relation to oxygen of these organisms when growing in a standardized complex medium containing glucose. Those species which are facultative would then be

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placed in the genus Staphylococcus and those species which are obligate aerobes would be assigned to the genus Micrococcus. To a limited extent the results would depend upon the constituents of the basal medium employed. For example, it has long been known that Staphylococcus pyogenes can grow aerobically but not anaerobically in a "synthetic" medium in the absence of uracil (Richardson, 1936).

It is interesting to note that Hucker (1924) stated, "the mass-forming cocci appear to form a group midway between the obligate aerobes and the anaerobes, and being such a transition group in respect to oxygen requirements, it is not unlikely that such a character will be found suitable for classification." However, he found no correlation between oxygen-requirements and sugar fermentations. This lack of correlation may well have been due to the methods employed. Oxygen requirements were determined by observing the nature of the growth in an agar stab culture and sugar fermentations were determined by observing acid production on agar slope cultures. It is probable that the use of agar shake cultures, or a comparison of aerobic and anaerobic growth in a broth medium, would show that many strains that Hucker designated as facultative are actually aerobic and many designated as microaerophilic are truly facultative.

Shaw, Stitt, and Cowan (1951) have proposed grouping the micrococci based upon acid production from glucose. Their failure to differentiate between fermentation and respiration may result in a less clear cut separation. Many of these organisms produce considerable ammonia from the nitrogenous constituents of the medium under aerobic conditions, thus tending to mask acid produced from the glucose. Examples are illustrated in Table 1. Only 4 of these strains are capable of true glucose fermentation. Some strains (e.g. 399) produce much acid aerobically although they are unable to metabolize glucose anaerobically. Strains such as 412 and 416 represent intermediate types. It is interesting to note that Shaw et al. studied most of these same strains and placed those that fermented glucose in their Subgroups 1 and 2, those which produced considerable acid aerobically but none anaerobically in Subgroup 3, and those which produced little or no acid aerobically in Subgroups 3 and 5. However, it is most doubtful that such a good correlation would exist if such a comparison were extended to more strains in Subgroups 1-4.

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Table 1

Glucose fermentation and respiration by a
collection of named strains of micrococci

Name	ATCC number	Final pH in Broth culture			Subgroup of Shaw et. al.
		Basal* (aerobic)	Glucose* (aerobic)	Glucose** (anaerobic)	
<i>M. epidermis</i>	155	7.9	5.3	5.1	2
<i>M. rhodochrous</i>	184	8.8	8.6	6.8	
<i>M. rubens</i>	186	8.5	6.9	6.8	4
<i>M. citreus</i>	395	8.9	8.7	6.8	
<i>M. luteus</i>	398	8.7	8.7	6.7	5
<i>M. varians</i>	399	8.9	5.0	6.6	3
<i>M. flavus</i>	400	8.4	7.7	6.6	5
<i>M. conglomeratus</i>	401	8.9	4.9	6.5	3
<i>M. freudenreichii</i>	407	8.5	8.4	6.5	5
<i>M. ureae</i>	408	8.4	8.2	6.7	5
<i>M. roseus</i>	412	9.0	7.1	6.6	4
<i>M. roseus</i>	416	8.8	7.6	6.7	4
<i>M. denitrificans</i> (L-450)		8.9	8.8	6.7	
<i>M. agilis</i>	998	8.9	7.5	6.8	5
<i>M. lysodeikticus</i>	4698	8.5	7.8	6.8	5
<i>M. candidus</i>	8425	7.4	4.6	4.3	
<i>M. candidans</i>	8456	8.2	7.9	6.8	5
<i>M. caseolyticus</i>	8460	8.0	5.0	5.0	1
<i>M. pyrogenes</i> (85320)		8.2	5.6	5.0	
Uninoculated control		7.1	6.9	6.8	

* The basal medium contained 1.0 per cent trypticase (BBL) and 0.5 per cent yeast extract (Difco). Ten ml of medium per 50-ml Erlenmeyer flask were inoculated and incubated for 10 days at 20°C.

** The medium for this series contained 1.0 per cent trypticase (BBL), 0.5 per cent yeast extract (Difco), 1.0 per cent glucose, 0.5 per cent NaCl, and 0.1 per cent K_2HPO_4 . Five-ml amounts in 16 mm test tubes were inoculated, covered with sterile vaspar, and incubated for 10 days at 20°C.

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To maintain the stability of these characteristics it would be advisable to carry stock cultures in media containing glucose. The obligate aerobes should be cultured on agar slopes or in shallow layers of broth, and the facultative organisms in ordinary tubes of broth media.

The taxonomy of these genera would best be served by defining valid species only on the basis of combinations of characteristics that occur with considerable frequency in strains isolated from different sources. This policy has fortunately been adhered to by most workers with this group of microorganisms. One should certainly refrain from establishing new species based on the study of only one or two strains, no matter how unique their characteristics may be. We also agree with Shaw et al. that it does not seem advisable to place strains that tend to form tetrads or regular packets into separate genera. They may represent distinct species within the genera Micrococcus and Staphylococcus.

CONCLUSIONS

Micrococcus luteus (Schroeter) Cohn is a recognizable species and ATCC culture 398 appears to be a satisfactory type culture. The ability to utilize ammoniacal nitrogen as a sole source of nitrogen is not a test applicable to this group of organisms because it cannot be studied independently of the energy metabolism and nutritional requirements for growth. The genera Micrococcus and Staphylococcus can be separated logically on the basis of their relation to oxygen in a standardized complex medium containing glucose. The former are obligate aerobes and the latter are facultative. Staphylococcus pyogenes is an extremely homogeneous species and the type culture suggested by Cowan, Shaw, and Williams (1954) appears quite satisfactory.

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