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STATUS OF THE GENUS CHROMOBACTERIUM

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Papers have been published during the past year on the status of the genus Chromobacterium by Leifson (2) (Morphological and physiological characteristics of the genus Chromobacterium); by Sneath (3) (Cultural and biochemical characteristics of the genus Chromobacterium); by Sneath (4) (The change from polar to peritrichous flagellation in Chromobacterium spp.); by Eltinge (1) (Nitrate reduction in the genus Chromobacterium) and by Sneath (5) (On conservation of the genus name and designation of the type species and type strains).

Detailed studies of the flagellation in this genus have been carried out by both Leifson and Sneath. They agree that all strains studied have a polar flagellum and may have in addition one or more lateral flagella. They also found that the lateral flagella have a shorter wave length and are more easily stained than the polar ones. Sneath concluded that both cultural conditions and age of the culture largely determined the presence or absence of lateral flagella. These were most abundant in young cultures on solid media and least abundant in broth cultures and in old cultures on solid media. Sneath also found that the two types of flagella differed in antigenic composition.

Either of two methods may be chosen for a study of the physiological differences between organisms belonging to a genus. By one method numerous strains may be assembled from culture collections and others isolated over a period of years, from as many localities as possible, and studied by as many tests as time and facilities permit. Or alternatively fewer cultures may be collected and intensively studied using many more differential criteria and tests. Both methods have value, and when critical studies of selected strains are combined with less comprehensive studies of a large number of strains, noteworthy differences should become apparent.

Leifson and Sneath made use of the second method in studies of 28 and 38 cultures respectively. Their cultures indicated that the genus could be divided into psychrophiles and mesophiles with a group of other characteristics common

to each of these groups. Eltinge also thought the genus should be divided on temperature until she obtained three cultures isolated from soil from the southern states which were mesophiles but had all other characteristics which are common to psychrophiles. She has also observed that a strain which usually produced good growth at 4° C might at times be unable to grow at 4° C and still not be able to grow at 37° C. She concluded that temperature does not seem to be a wholly satisfactory criterion upon which to base differentiation of species.

Eltinge in a study of 82 cultures concluded that the genus may be divided into three groups of strains on the basis of ability to reduce nitrate. One group reduces nitrate completely either with or without the production of visible gas. This group includes the psychrophiles and the three mesophiles isolated from southern soil. Strains in the second group reduce nitrate to nitrite but are not able to reduce nitrite. Those in the third group are unable to reduce either nitrate or nitrite. Based on other common characteristics, the second and third groups may well be combined. Results reported by Sneath are not completely in harmony with this grouping on the basis of nitrate reduction. He found most mesophiles could utilize nitrite slightly and that an occasional strain could do so rapidly.

Leifson reported that some of the mesophiles, which oxidized glucose, were also able to ferment glucose and a few other carbohydrates anaerobically. This finding has been confirmed by both Sneath and Eltinge. Leifson would separate this group of fermenters from other mesophiles which can oxidize only. This finding would make plausible the recognition of two species in the mesophilic group.

When tests on carbohydrate utilization as reported respectively by Leifson, Sneath and Eltinge are compared, some variation in results are evident. These could doubtless be caused by adaptations within the strain, perhaps permanent or transitory caused by environmental conditions. In the six years the writer has worked with members of this genus she has found many strains capable of varied adaptation. Sometimes cultures of presumably identical strains obtained from different individuals will differ in one or more characters when tested.

Another criterion available for separation of species within the genus is utilization of maltose. So far, this seems a dependable distinguishing characteristic. Strains

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able to utilize maltose include all the psychrophiles and the three strains from southern soil. These are also the strains which completely reduce nitrate. All other strains seem unable to use maltose. However, recognizing how much variation there may be in utilization of carbohydrates between strains, a recognition of species within the genus based solely upon the utilization of a specific carbohydrate does not seem wise.

The three authors agree that the genus name for the violet organisms with pigment soluble in ethanol but not in chloroform or water should be Chromobacterium. Leifson, Sneath and Eltinge agree that in Chromobacterium there should be recognized at least two species, but they do not agree as to the criteria. Leifson and Sneath would divide the genus on temperature and Eltinge upon the utilization of nitrate. Sneath would make two species. He has proposed the name Chromobacterium violaceum Bergonzini 1880 for mesophilic strains and Chromobacterium lividum (Eisenberg) Holland for psychrophilic strains. Sneath (5) recommended that the type species be a mesophilic one.

Leifson would recognize three species. He proposes that the name Chromobacterium violaceum (Schroeter) Bergonzini be assigned to the psychrophilic strains. He would make two species of the mesophilic strains based on differences in carbohydrate utilization. For strains able to ferment he proposed the name Chromobacterium manilae comb. nov. and for those able to oxidize only the name Chromobacterium laurentium comb. nov.

The author would make two species. The name Chromobacterium violaceum (Schroeter) Bergonzini is proposed for strains able to reduce nitrate completely and which usually grow at 4° C if a psychrophile is chosen as the type species, otherwise the name C. lividum (Eisenberg) Holland is proposed for the species. She would also recommend in addition to the typical variety, a named variety for the strains able to grow at 37° C and not at 4° C and suggests the new name Chromobacterium violaceum var. mesophilum. For strains which fail to reduce nitrate or reduce it only to nitrite, which grow at 37° C or higher and which usually ferment some carbohydrates she proposes the name Chromobacterium janthinum (Zopf) Holland; if the mesophile is chosen as the type species she proposes instead the name C. violaceum Bergonzini. It may be that two varieties should be recognized, one based upon ability to reduce nitrates to nitrites. Another possibility

is the recognition of two varieties based upon ability to ferment carbohydrates.

The name of the type species of a genus must under the rules be the name of a species included by the author in the genus when first proposed. One species only was named by Bergonzini in the original paper. The name Chromobacterium violaceum Bergonzini must therefore be applied to the type species. The question arises as to whether this name shall be applied to a mesophilic or a psychrophilic strain. What names should be applied to other species is also debatable.

Sneath proposes that the type species be fixed as a mesophile. The author agrees with him. One reason for this is that a mesophilic strain lasts longer under cultivation than a psychrophilic one and therefore, is more often found in culture collections. If a mesophilic strain is chosen as the neotype culture or strain, then the strain MK of Sneath (NCTC 9757, ATCC 12472) would be suitable. If a psychrophilic strain is chosen as the neotype, then the strain HB of Sneath (NCTC 9796, ATCC 12473) is suitable.

The views of Leifson, Sneath and the author as to the differential characters and species names to be applied in the genus Chromobacterium may be summarized by the following three keys.

Chromobacterium

Key to species according to Leifson (2).

- I. No growth at 37° C. Oxidative metabolism of carbohydrates. Acid from glucose, levulose, mannose, arabinose, xylose, maltose, mannitol, and sorbitol. Sucrose variable. Lactose, salicin and dulcitol negative. Nitrite produced from nitrate. Utilization of citrate positive. Gelatin liquefaction weak or negative. Indole and urea negative.

1. Chromobacterium violaceum (Schroeter) Bergonzini.

II. Growth at 37° C.

- A. Fermentative metabolism of carbohydrates. Acid without gas from glucose, levulose, mannose and inulin. Sucrose variable. Arabinose, xylose, maltose, mannitol, sorbitol, lactose, salicin and dulcitol negative. Nitrate reduced to nitrite or negative. Gelatin liquefied. Citrate utilization variable. Indole and urea negative.

2. Chromobacterium manilae Leifson.

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B. Oxidative metabolism of carbohydrates. Acid from glucose and levulose. Mannose, arabinose, xylose, lactose, sucrose, maltose, inulin, mannitol, sorbitol, salicin and dulcitol negative. Nitrate reduced to nitrite. Gelatin liquefied. Citrate negative or slowly metabolized. Indole and urea negative.

3. Chromobacterium laurentium Leifson.

Chromobacterium

Differentiation of species according to Sneath (3, 4, 5).

I. Strains which grow at 4° C but not at 37° C. Size large, fat scanty, proteolysis slight, haemolysis slight, anaerobic growth scanty or absent, citrate utilized rapidly, HCN not detected, egg yolk reaction negative, oxidizes glucose, does not break down trehalose, hydrolyzes aesculin.

1. Chromobacterium lividum (Eisenberg 1891) Holland 1920.

II. Strains which grow at 37° C but not at 4° C. Size small, fat usually abundant, proteolysis marked, haemolysis marked, anaerobic growth usually good, utilize citrate slowly, produce HCN, egg yolk reaction positive, acid from trehalose, aesculin not hydrolyzed, attack on glucose usually fermentative.

2. Chromobacterium violaceum Bergonzini 1880.

Chromobacterium

Key to species according to Eltinge.

(1) If a psychrophile is chosen as the type species.

I. Nitrate reduced completely with or without the production of visible gas. Oxidize glucose and maltose and usually xylose and arabinose. May oxidize sucrose. Gelatin slowly liquefied. Usually good growth at 4° C with no growth at 37° C.

1. Chromobacterium violaceum (Schroeter) Bergonzini.

A. Strains which grow at 4° C but not at 37° C. Oxidize glucose and maltose and usually xylose and arabinose. May oxidize sucrose.

1a. Subspecies (var.) violaceum var. nov.

B. Strains which grow at 37° C but not at 4° C. Oxidize glucose and maltose and occasionally fructose.

1b. Subspecies (var.) mesophilum var. nov.

II. Nitrate reduced to nitrite or not at all. Oxidize glucose, frequently fructose and occasionally sucrose. May ferment glucose, sucrose, fructose and arabinose. All grow well at 37°, many at 40° and a few at 42° C. No growth at 4° C. Gelatin rapidly liquefied.

2. C. janthinum (Zopf) Holland.

A. Strains which ferment glucose and sucrose, frequently fructose and occasionally arabinose. Oxidize glucose, frequently fructose and occasionally sucrose. May or may not reduce nitrate to nitrite.

2a. Strains which reduce nitrate to nitrite--subspecies (var.) janthinum var. nov.

2b. Strains which do not reduce nitrate--subspecies (var.) anitritum var. nov.

B. Strains which oxidize glucose and usually fructose but do not ferment carbohydrates. Nitrates reduced to nitrites.

2c. Subspecies (var.) purpureum var. nov.

(2) If a mesophile is chosen as the type species.

I. Nitrate reduced completely with or without the production of visible gas. Oxidize glucose and maltose and usually xylose and arabinose. May oxidize sucrose. Gelatin slowly liquefied. Usually good growth at 4° C with no growth at 37° C.

1. Chromobacterium lividum (Eisenberg 1891) Holland 1920.

A. Strains which grow at 4° C but not at 37° C. Oxidize glucose and maltose and usually xylose and arabinose. May oxidize sucrose.

1a. Subspecies (var.) lividum var. nov.

B. Strains which grow at 37° C but not at 4° C. Oxidize glucose and maltose and occasionally fructose.

1b. Subspecies (var.) mesophilum var. nov.

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II. Nitrate reduced to nitrite or not at all. Oxidize glucose, frequently fructose and occasionally sucrose. May ferment glucose, sucrose, fructose and arabinose. All grow well at 37° C, many at 40° C and a few at 42° C. No growth at 4° C. Gelatin rapidly liquefied.

2. C. violaceum Bergonzini 1880.

A. Strains which ferment glucose and sucrose, frequently fructose and occasionally arabinose. Oxidize glucose, frequently fructose and occasionally sucrose. May or may not reduce nitrate to nitrite.

2a. Strains which reduce nitrate to nitrite--sub-species (var.) violaceum var. nov.

2b. Strains which do not reduce nitrate--subspecies (var.) anitritum var. nov.

B. Strains which oxidize glucose and usually fructose but do not ferment carbohydrates. Nitrates reduced to nitrites.

2c. Subspecies (var.) purpureum var. nov.

SUMMARY

Chromobacterium (like many other bacterial genera) is difficult to divide into species because of the considerable differences between strains. In conclusion it should be pointed out that neither temperature nor reduction of nitrate seem to be the perfect criterion for delimitation of species. There are intermediates. However, most strains may easily be separated into two groups either on the basis of temperature or on nitrate reduction. When 82 strains were compared, a division based on nitrate reduction seemed to give more consistent results than one based on temperature.

All three authors agree on the use of Chromobacterium violaceum to designate one of the species. Two agree that this name should be given to a psychrophile, the third to a mesophile.

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