

## MISCELANEA

### Systematic study of some aerobic sporulated bacteria \*

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#### SUMMARY

This paper reports the results of a systematic study of sporulating aerobic nonpathogenic bacteria.

The material investigated consisted of 206 strains, isolated by the author herself or obtained through the courtesy of several museums of bacteriology, as stated in the list of annex 1, where number, denomination, origin and date of isolation are included. Of these strains belong to the well-known species *B. subtilis* Cohn, 1872; *B. vulgatus* (Flügge) Trevisan, 1889; *B. mesentericus* (Flügge) Migula, 1900; *B. globigii* (Globig) Migula, 1900; *B. atterimus* Lehman and Neumann, 1896; *B. niger* (Gorini) Migula, 1900; *B. simplex* Gottheil, 1901; *B. lautus* Matchelor, 1919; *B. tritus* Batchelor, 1919; *B. mycoides* Flügge, 1886; *B. adhaerens* Laubach, 1916; *B. cereus* Frankland, 1887; *B. megatherium* De Bary, 1884; *B. petasites* Gottheil, 1901; *B. circulans* Jordan, 1890; *B. fusiformis* Gottheil, 1901; *B. pseudotetanicus* (Krusse) Migula, 1900; *B. polymyxa* (Prazmowsky) Migula, 1900; *B. asterosporus* (Meyer) Migula, 1900; *B. laterosporus* Laubach, 1916; to new varieties belong: *B. cereus* var. *albolactis* n. comb.; *B. polymyxa* var. *acetoethylicum* n. comb.; and to new species belong: *B. spinosporus*; *B. biacutum*; *B. indifferens*; *B. pellucidus*; *B. ubicuitarius*; *B. mutabilis*; *B. platus*; and *B. naviformis* (the specific denomination denoting some of the most striking characteristics observed in each of the last eight species).

The characterization of the species and varieties is based on their morphologic study [culture by pen («federstrichkultur») and stained preparations]; on their characteristics of growth (agar plate and agar streak culture, broth, gelatin stab and potato culture); and on physiological investigations (i. e. relations to temperature, thermoresistance of the spores, relations to medium reaction, chromogenesis, relation to free oxygen, indole production, production of hydrogen sulfide, action on milk, reduction of nitrates, acetyl-methyl-carbinol production, action on fats, heavily glucosated agar culture, hydrolysis of starch and fermentation of carbohydrates and like substances). All these tests have been carried out following the techniques which, according to their diffusion in bibliography, are described in the chapter on methods of investigation (where the isolation technique is also found) or else they are found in annex II.

In this way author has been able to control the importance that those methods have in the ultimate classification. In fact, the comparative study of the morphologic and cultural data have enabled the author to determine accurately 17 species, while for determination of the remaining 13 physiological investigations have been found indispensable.

Morphology, characteristics of culture, physiology and fermentation of carbohydrates and like substances of the 30 species have been summarized in tables. As a corollary a determinative key is given, founded on specific characteristics

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of simpler determination. Then follow nine general bacteriological considerations with regard to *a*) the existence of a second generation of bacteria in the same preparation of culture by pen; *b*) the duration of motility of bacteria in preparations of this kind; *c*) the likelihood of clostridia being transitional forms; *d*) the production of clostridios and plectridia in relation to the behaviour of the generating species, the temperature, medium reaction and enzymatic capacity; *e*) specific granulations; *f*) motility in relation to sporulation; *g*) adherence of growths; *h*) modification of some characteristics of the growths as a sign of bacterial variation; and *i*) pigment formation.

The detailed systematic study is preceded by an introduction stating the aim of the work, the extent of the present contribution and the importance of reintegrating Bacteriology to its legitimate condition of prominent branch of Natural Sciences. Then follow a brief historical outline, some critical comments on the real worth of the bibliography recorded in literature and, finally, the diagnosis of the genus *Bacillus*. The systematic study is followed by critical remarks on nomenclature regarding: 1 *B. subtilis*; 2 *B. cereus* Frankland confronted with *B. albolactis* Migula and 3 *B. acetoaethylicum* Northrop, *B. asterosporus* Meyer and *B. macerans* Schardinger confronted with *B. polymyxa* (Prazmowsky) Migula. Basing her statements on bibliography and laboratory research (the latter not being included in the present paper), the author comes to the conclusion that the creation of the genus *Bacillus* and its type species are wrongly established; that the solution of such an important problem should be the object of some international congress of microbiology in the near future and that a systematic review of bacteriologic nomenclature should be realized as is commonly done with other Natural Sciences. Regarding the second question the author gives the reasons why she considers *B. albolactis* as a variety of *B. cereus*; while regarding the third question *B. asterosporus* is considered as a good species and *B. acetoaethylicum* as a variety of *B. polymyxa*.

With a view to their diffusion tables of the excellent synopsis published by Chester appear in annex IV. To corroborate some of the critical comments already mentioned the determinating keys of Chester, Lehmann and Neumann and Bergey are added in annex V.

The paper is illustrated with 30 microphotographies of bacteria in preparations of cultures by pen; 30 photographs of colonies from agar plate cultures and 30 drawings using Abbe camera.

#### CONCLUSIONS

1. Lindner methods of culture by pen and by adhesion in hanging drop, render great help in the morphologic study of microbes and are almost enough to determine a great number of the bacterial species studied.
2. Determination of sporulating bacteria based on their size is very practical, in spite of its being artificial, when used for purposes of comparison.
3. The designation of some species described in current literature has not always been found to be accurate. In some instances the differences observed have been so slight that they have been regarded as simple varieties of the species already described. Thus, *B. albolactis*, a variety of *B. cereus* and *B. acetoaethylicum* a variety of *B. polymyxa*.
4. Most species could be classified both for their morphology and characteristics of culture. Others required besides determination of some physiological characteristics.
5. The 206 strains studied have been grouped into 28 different species and 2 varieties. Eight species have been classified anew because some of their fundamental characteristics did not correspond to those of any species so far described.