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SOME ASPECTS OF THE TAXONOMY OF THERMOPHILIC BACILLI

Helge H. G. Gyllenberg

Department of Microbiology, University of Helsinki, Finland

The first question arising in connection with thermophilic bacteria is: What bacteria fulfill the requirements usually given for thermophilic organisms. In the suborder *Eubacteriineae* there are in fact, apart from the thermophilic group of aerobic sporeformers, only a few species characterised by thermophilic behaviour: *Lactobacillus thermophilus*, *Clostridium thermosaccharolyticum*, and *C. nigrificans*. In addition to these species, bacteria with optima between 37 and 50°C have often been termed thermophiles. This is true especially in the field of dairy bacteriology. Of this at least one illustration exists in the current edition of Bergey's Manual, viz. *Streptococcus thermophilus* Orla-Jensen. It would seem more correct if organisms that grow readily above 50°C, but whose optima lie below this point, were termed *thermotolerant mesophiles*. This term was proposed by Schillinger in 1898 with this significance.

As regards *Lactobacillus thermophilus*, it can be said that this organism is closely related to the homologous group of other lactobacilli, with their distinct thermotolerant character. Hence it may be a ques-

tion of interpretation as to whether it is correct to classify this organism also as having the same characteristics of thermal temperature relationship. As to the clostridia, it may be observed that, although anaerobic cellulose decomposition is well known, it has thus far been impossible adequately to characterize the organisms causing this fermentation. It seems probable, however, that our knowledge of this group will be broadened in the immediate future. In spite of the possibility of descriptions of new thermophilic *Clostridium* species, it seems improbable that the thermophilic clostridia will constitute a definitely delimited group in any respect.

It is from this point of view that thermophilic bacilli are of special interest. At first glance the opinion that these organisms constitute a physiologically unique group may seem well founded. It has been proposed by some earlier authors that these thermophilic bacteria should be placed in a special genus (e.g. *Thermobacillus* Feirer). Although such a grouping would facilitate their differentiation from the mesophilic species, it must be born in mind that the ability to

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form endospores does not constitute the sole resemblance between mesophilic and thermophilic aerobic spore-formers. In fact there are a number of morphological and physiological characteristics in common and a comparison of the mesophilic and thermophilic bacilli reveal some interesting facts.

As a starting point for such comparisons it may be emphasized that thermotolerance is characteristic of a number of mesophilic bacilli. Further, an identical basis for preliminary differentiation among the several species may be employed, particularly, location and form of spores, shape of sporangia, and diameter of rods. The present author has attempted to determine the identity of certain thermophilic *Bacillus* strains by employing the current key to this group of bacteria. This proved unsuccessful, apparently in part because of the very inadequate data recorded for some thermophilic species. Thereupon a key to the mesophilic species was employed both with these strains and with the thermophilic species described. By this technique the relationship of the strains studied to the thermophilic species patterns could be established in most cases. This method of identification also gave an interesting picture of the relation between the described mesophilic and thermophilic species.

The author found that 4 strains isolated by him were closely related to *Bacillus subtilis*. These strains could be characterized as thermotolerant, even though 2 of them grew at 65°C, but quite poorly. The remainder which did not grow at 65°C grew well at 57°C. One strain of each group failed to grow at 18°C. It seems that all of these strains were identical with *B. subtilis*, although they possessed an exceptional heat tolerance. On the other hand, these strains also seemed to be related to *B. thermodiastaticus*, differing most distinctly in their ability to grow at low temperatures. Variants of this type, capable of growth at 40°C, are, however, known (*Therobacillus reductans* Feirer). Therefore it may be postulated that thermophilic, including stenothermal, variants of *B. subtilis* may exist.

On the basis of temperature relationships *B. kaustophilus* and *B. thermoindifferens* are more closely related to *B. subtilis* than is *B. thermodiastaticus*. They differ, however, from *B. subtilis* in certain physiological properties.

These species seem to be related also to *B. firmus* and *B. lentus* in some respects. A strain resembling *B. thermoindifferens*, studied by the author, was in fact sensitive to acidity in the medium.

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As to the second group of thermophiles (*B. cylindricus*, *B. robustus*, and *B. calidolactis*) no distinct points of connection seem to exist with the corresponding group of mesophiles (*B. megaterium*, *B. cereus*). Among the strains studied by the author there were, however, 3 strains closely resembling *B. cereus* morphologically as well as physiologically. One of these 3 strains grew at temperatures from 18 to 57°C. The other two failed to grow at 28°, but grew well at 65°C. One of these, however, was incapable of hydrolysing starch.

The most noteworthy characteristic of the organisms of the first subgroup of mesophilic bacilli with distinctly swollen sporangia is the gaseous fermentation of carbohydrates (*B. polymyxa*, *B. macerans*). One thermophilic species able to produce gas from carbohydrates is known, but this (*B. thermamylolyticus*) differs distinctly in its morphology from the gas-forming mesophiles.

The next group of mesophiles (sporangia swollen, no gas from carbohydrates, growing on ordinary media) includes the species *B. circulans*, *B. alvei*, *B. laterosporus* and *B. brevis*. To these correspond morphologically the thermophilic species *B. michaelisii*, *B. thermotranslucens*, *B. thermocellulolyticus*, *B. thermoalimentophilus*, *B. thermoliquefaciens* and *B. tos-*

tus. Apart from the properties mentioned, it is very difficult to draw further conclusions concerning the relations of the species, due to the scanty data on the physiological properties of the thermophilic species. The author, however, has studied an organism which was related in most respects to *B. circulans*. This strain grew in the 39 to 65°C range, with a distinct optimum above 50°. Also *B. thermotranslucens*, and more distinctly *Thermobacillus linearis* Feirer, can be considered as related to *B. circulans*, but it is said that these fail to liquefy gelatin or reduce nitrates. Between *B. laterosporus* and *B. thermoalimentophilus* there seems to be some resemblance. Also *B. thermoliquefaciens* may be related to *B. laterosporus*. Four of the author's strains seemed to be related to *B. laterosporus*. All of these grew poorly at 65°, two grew at 28°, but none of them at 18°C. Three strains differed in that they lacked the ability to liquefy gelatin, and one of these in addition failed to reduce nitrates. Two of the author's strains were related to *B. brevis*. Both of these grew throughout the 18 to 65°C range. Some strains described by other authors can also be considered as related to *B. brevis*. This seems likely of *Bacillus nondiastaticus* Bergey, *Thermobacillus vulgaris* Feirer, and *Thermobacillus violaceus* Feirer. Feirer's strains, however, as stated in

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descriptions fail to reduce nitrates or to liquefy gelatin.

On the basis of the comparison, outlined above it can be stated that the relationship between mesophilic and thermophilic bacilli may be closer than has commonly been considered. This aspect of the taxonomic position of the thermophiles has perhaps been by-passed since the temperature relations have appeared as more characteristic features than the other physiological properties. A noticeable question, and an object for further investigation may be, however, to establish how close are the relations in

fact between both these groups of bacilli. It does not seem impossible that some of the thermophilic bacilli, when further investigated, may well be found to be included with known mesophilic species. If this were to be proved, the question of revising the current sub-grouping of the genus *Bacillus* (into mesophiles and thermophiles) will be opportune. The most important subject of investigation, however, is to broaden our knowledge of the physiological properties of known thermophilic species in order to diagram their mutual relationships, and to facilitate their determination.