STUDIES ON AEROBIC SPORE-BEARING NON-PATHOGENIC[1] BACTERIA

PART I

From the Laboratory of Hygiene and Bacteriology, Johns Hopkins University

INTRODUCTION

BY W. W. FORD

One of the most important problems of modern hygiene is the identification and classification of the bacteria in our environment. Microörganisms of various kinds exist everywhere in nature and influence profoundly all sorts of substances which affect man's physical condition. This is true of food-stuffs in general and especially true of milk which is markedly altered in its chemical composition by the bacteria which multiply in it. The microörganisms in our environment are of various sorts, pigmented bacteria, spore-bearing bacteria, yeasts, moulds, etc. Some of these forms are identified without great difficulty but our knowledge of the spore-bearing bacteria is still in a state of chaos. The reason for this lack of knowledge is not far to seek. The science of bacteriology developed primarily among physicians whose interest naturally lay in the disease-producing properties of the various parasites which infect man and the animals. Non-pathogenic bacteria were of importance chiefly as laboratory contaminations to be avoided. With the development of industrial bacteriology those species were again most carefully studied which seemed to serve some distinct purpose in nature, as for example, the nitrifying bacteria of the

[1] The term "non-pathogenic" is used here in the sense of "lacking in disease-producing properties." Many spore-bearing bacteria are at times pathogenic to small animals and instances are reported in which they may produce inflammatory reactions when vegetating on mucous surfaces. The organisms here described are however in no instances capable of producing definite diseases

soil and the lactic acid bacteria in milk. In consequence the bacteria found in nature which seem to be lacking in any definite function have been largely neglected. At various times many species of spore-bearing organisms have been described and recorded in the literature and in many instances these cultures have been kept alive in laboratories both in Europe and in America. It would seem an easy task therefore to collect the spore-bearing bacteria from different institutions, make a careful study of their properties and arrive at some conclusion as to their identity and classification, just as is done with the pathogenic species. This method of solving the difficulty is open to serious objections, however, and has not thus far proved of great value. In the first place the descriptions originally given of many of these species are meager and the original cultures have not been saved. In consequence the literature of bacteriology is thickly strewn with names of spore-bearing organisms which have absolutely no meaning. The term *Bacillus subtilis* for instance is applied to almost any large microorganism which forms spores readily and grows abundantly on artificial media, and cultures identified as *Bacillus subtilis* by different bacteriologists are often found to have little or nothing in common. Again the cultures which have been kept alive have in many instances so changed in character as no longer to give the reactions orginally described. Thus Migula (1897) found that of some six hundred cultures obtained by him from the laboratories in Germany only a small number had the characteristics first ascribed to them. Finally, pure strains of spore-bearing bacteria are more difficult to keep in direct descent in the laboratory than are other species. When cultures become contaminated it frequently happens that the contaminating species is picked up from the plates made to purify the strain and carried on as the original. This has happened a number of times in our own laboratory during the past few years and in consequence we have become very sceptical of the value of any conclusions based upon a comparison of existing stock cultures.

A number of years ago an attempt was made, in the laboratory of Dr. Adami in Montreal (Ford, 1903) to separate and classify

the sporulating organisms by the use of carbohydrates. The result of this work was not entirely satisfactory because of the difficulty of establishing the fundamental species from which to build up our system of classification. During the past few years, however, a number of very valuable papers on spore-bearing bacteria have appeared in the literature and have cleared up some of the most difficult points. Of especial importance is the work of Meyer (1903) and his collaborators, Gottheil (1901) and Neide (1904) in Germany, and the work of Chester (1903) in this country. As a result of the efforts of these authors we now have accurate descriptions and definite means of identification of a small number of our most common spore-bearing species.

Some four years ago a large number of spore-bearing bacteria was obtained from raw milk and from milk heated to various temperatures from 60° to 100° and so much difficulty was encountered in their identification that it seemed as if the time was ripe for a more extensive investigation of the subject, based upon the work above referred to. The problem was first undertaken by Mr. Lawrence and myself with the organisms from milk. After a working basis had been obtained for the classification of these species a study of the spore-bearing bacteria of water was undertaken by Dr. Laubach, and of the soil by Dr. Laubach and Mr. Rice with the object of testing the classification already adopted for milk bacteria and of adding to it such species as had not previously been encountered in our work. Finally stock cultures of well-known species were obtained from the Kral collection in Vienna, the Winslow collection in the American Museum in New York, from the laboratories of hygiene of the University of Pennsylvania, and of the University of Chicago, and the bacteriological laboratory of the Sheffield Scientific School, and our cultures were compared with them. From the start of the work however, our object has been to establish clearly the different types of spore-bearing organisms in our own laboratory and then to link these types up with types already established by other observers. Altogether over 1700 cultures have been studied from various sources, milk, soil, dust,

water, intestinal contents and contaminated plates. From this number we have obtained 28 distinct types of which 22 are clearly to be identified as well known species, 2 are distinct varieties of old types and 4 are evidently new species. In general our aim has been to clarify our knowledge in regard to old species and not to establish new types except when our isolations showed certain characteristics not already referred to in the literature and of distinguishing importance.

The media employed in this work were the standard media of the laboratory. A great deal of emphasis was laid upon the reactions with gelatin, with litmus milk, with glucose, saccharose, and lactose broth, with glucose litmus agar and with Loeffler's blood serum. The morphology was studied from smears made from plain and glucose agar cultures 6 to 8 hours old and 22 to 24 hours old, and from cultures 1 to 2 weeks old, the organisms being always stained with Gentian violet. The same preparations were used later for measurements and for illustrations. The method of sporulation and the size, shape and position of the spore were observed with great care. A study of the spore wall, and its differentiation into the exine and intine of Gottheil and Chester, while interesting and important, proved of little help in classification. The method of spore-germination was likewise found relatively valueless. Nearly every type of spore-germination could eventually be found with most species and our observations were so inconstant as not to furnish any basis for classification. Micro-chemical reactions, while undoubtedly of great value, could not be worked out with any degree of thoroughness and were eventually discarded. Careful observations were made upon the thermal death points which were established with broth cultures subjected to various degrees of temperature in the Arnold sterilizer and in the autoclave. In general our classification may be said to rest upon morphological and tinctorial properties, spore-formation and cultural reactions. How valuable our results are can only be determined by the extent to which other workers may be able to utilize this classification in subsequent investigations.