Microbiology. — *Spore-formation by the sulfate-reducing vibrio.* By R. L. STARKEY. (Communicated by Prof. A. J. KLUYVER).

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Microbial reduction of sulfate to sulfide is a reaction characteristic of a very small group of closely related bacteria which are strictly anaerobic vibrios having the same morphological appearance. The first of these organisms was described by BEIJERINCK (1) in 1895 as *Spirillum desulfuricans,* more recently called *Vibrio desulfuricans.* Thermophilic and halophilic cultures have been obtained, but BAARS (2) has shown that these are not distinct species, but rather adapted strains of the organism which grows at ordinary temperatures and in the absence of appreciable amounts of salt. BAARS described one additional species (*Vibrio Rübentschickii*) and an additional variety of this species. Except for some differences in the utilization of fatty acids and certain carbohydrates, the three cultures were alike.

The organisms are not alone of interest due to their specific and unusual transformation of sulfate. By reason of their general distribution in nature and their activity in the reduction of sulfate in mud, soil, sewage, and water, they are of great importance in nature.

In the course of observations of cultures of sulfate-reducing bacteria, bacterial spores were commonly encountered when the cultures were incubated at 55° C. All attemps to eliminate the spores failed. The cultures grew well as surface colonies on anaerobic plates of lactate agar, nutrient agar, and peptone-dextrose agar to which sulfate was added. Repeated transfer and replating of well separated colonies from such plates yielded the same type of cells. These were quite large vibrios varying considerably in size, as noted by BAARS, but commonly measuring $3.5 \times 0.7\ \mu$ in young cultures. As the cultures became older, granules appeared in the cells and many of the cells were either relatively short or much longer than the original ones, even with complete spirals instead of the more common short arc. Young cells show little progressive motility but spin and twist actively. The incubation period at which spores occur in the cells is variable. In solution media at 55° C., they are most commonly observed within one or two days. Spore-formation is more readily detected in colonies on anaerobic plates of peptone-dextrose agar containing sulfate or in agar stabs of this medium kept under pyrogallol. On such media, sporulating cells may be numerous even after incubation for 3 to 5 days. The spores are highly refractive, oval, and relatively large bodies measuring about $1.7 \times 1.3\ \mu$. They are located sub-terminally in the cells and cause the cells

to swell to such an extent that they may lose their curved shape (fig. 1).

The spores are very resistant to high temperatures. Both cultures in the lactate solution and in agar media have been heated at 99°—100° C. for an hour without destroying the spores. This is the highest temperature used and the longest time any of the cultures have been heated and in none of these cases where spores have been observed have they failed to resist the temperature of boiling water. This explains the observation of BAARS that the thermophilic strains survived exposure to 80° C. for several hours.

When the lactate medium is inoculated with sewage, canal mud, soil, or similar materials and then incubated at 30° C., rather large vibrios appear similar to the cells which develop at 55° C. At this lower temperature spores are also produced. However, upon repeated transfer, such cells disappear completely and in their place there appear thin rapidly moving spirals and small vibrios (fig. 2). These cells make rapid progressive movement and do not show the spinning and twisting movement of the larger 55° vibrios. These are the cells which have been previously described as the more characteristic cells of *Vibrio desulfuricans*. Spores have never been found in cells of this type.

Spores of the sulfate-reducing bacteria are produced in nature as is indicated by the fact that the sulfate-reducing bacteria will grow from mud, soil, etc. that have heen heated before inoculation. Samples of ditch mud, canal mud, sewage and soil were suspended in sterile lactate solution and heated at various temperatures including 99°—100° C. for 30 minutes and then inoculated into the lactate medium and kept under anaerobic conditions at both 30° and 55° C. The sulfate reducers developed in all of the 56 solutions incubated at 55° C. and in all but two of the 56 solutions at 30° C. These two were from the fourteen solutions inoculated with garden soil which apparently contained fewer sulfate-reducers than the other materials. In all of the solutions, whether at 55° of 30° C., the first organisms to appear are sporulating vibrios but, while this type of cell persists in subcultures at 55° C., it is replaced by the smaller asporogenous vibrios and spirials at 30° after the first few transfers. It is therefore apparent that the sporulating cells actually change to the typically non-spore-forming small cells. Had the inocula not been heated, it might be presumed that both sporulating and asporogenous cultures existed together and that the former became overgrown by the latter and thus disappeared in time. However, the inocula were heated sufficiently to destroy all vegetative cells of the sulfate reducers and consequently the cells which developed originated from spores.

Many experiments have been performed to indicate that the sporulating cells persist at 55° C. while only asporogenous cells occur in the lactate medium at 30° after a few transfers. One example may suffice. Pure cultures which had been grown at 30° C. for several weeks and contained only

the small vibrios, were cultivated in lactate solution at higher temperatures by stages as performed by BAARS (2). They were first grown for several transfers at 35°, then transfered to 40°, then in a similar way to 45°, 50°, and 55° C. Actively growing cultures were thus obtained at each temperature for each of the cultures originally started from 30° C. In a similar manner, pure cultures of the sporulating type which had been grown for some weeks at 55° C., were inoculated at lower temperatures until active cultures were obtained at each of the six temperatures. Morphological examinations were made at each transfer and considerable changes were noted. After several transfers at each temperature, the cultures became stabilized. The most striking characteristic was the persistence of the non-sporulating small vibrios and spirals at 30°, 35°, and 40° whether the cultures had originated from 30° or 55° C. On the other hand, the cultures at 45°, 50° and 55° C. were all the larger vibrios which produced spores, even though they had started from asporogenous small vibrios at 30° C. After the cultures had become stabilized morphologically, they were tested for heat resistance. Portions of each of the solution cultures at each temperature were heated at 60° C. and 99°—100° for 10 and 30 minute periods. Only an occasional culture which had been grown at 30°, 35°, or 40° C. developed after being heated at 60° C. (5 of 48 inoculations). Furthermore none of the 48 solutions inoculated with cultures heated at 99°—100° C. for 10 or 30 minutes showed evidence of any growth. On the other hand, there was active development of the sulfate reducers in all but one of the 96 solutions inoculated with material from cultures grown at 45°, 50°, and 55° C. and then heated at 60° or 99°—100° for ten or thirty minutes. It thus seems apparent that the sporulating cultures can give rise to the asporogenous cultures and the non-sporulating can change to spore-formers. Temperature is the only factor which has been noted to induce these changes, but it seems likely that other factors are involved since the organism produces spores in its natural environment.

The cells are Gram-negative under all conditions, even the sporulating cells show no tendency to retain the stain.

There are a few isolated reports of sporulation in vibrios in the older literature, but none of these seem to have been verified. There is no indication in recent bacteriological literature that sporulation is recognized as a characteristic of any vibrio. It would therefore appear that this is the first decisive evidence of spore formation in this group of bacteria. It is not a new organism and all of the previous observers agree that the cells are typical of those commonly known as vibrios at the present time. Spore-formation has been verified by microscopic observation of both stained and unstained preparations and by tests of heat tolerance. The new generic name *Sporovibrio* is proposed for the sporulating curved anaerobic organisms of vibrio shape. The type species would be the strictly anaerobic, Gram-negative, sulfate-reducing organism *Sporovibrio desulfuricans.*

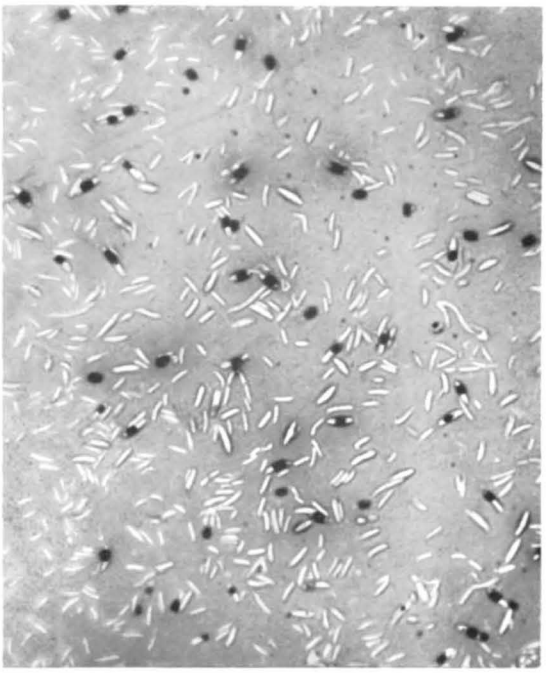

Fig. 1. Sporulating cells of the sulfate-reducing vibrio; from culture grown at 45° C.; modified Dorner stain. × 925.

Fig. 2. Small asporogenous vibrios and short spirals from culture grown at 30° C.; stained with gentian violet. × 925.

SUMMARY.

The specific sulfate-reducing organism known as *Vibrio desulfuricans*, Beij. produces spores which are very tolerant to high temperatures. It produces spores under natural conditions and spore-formation persists under laboratory conditions in cultures incubated at 45° to 55° C. Spore-formation disappears when the organism is cultivated for some time below 40° C. The change from sporulating to non-sporulating cultures and from asporogenous to sporulating cultures is reversible and can be effected by modifying the temperature of incubation using the same medium. The generic name *Sporovibrio* is proposed for the anaerobic sporulating vibrios with the type species *Sporovibrio desulfuricans* Beij., nov. comb.

A full report of these observations will soon be published elsewhere.

LITERATURE.

1. BEIJERINCK, M. W. 1895. Cent. f. Bakt., Abt. II, 1 : 1—9, 49—59, 104—114.
2. BAARS, J. K. 1930. Over sulfaatreductie door bacteriën. Dissertation, Delft.

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