

A MANUAL

OF

BACTERIOLOGY

BY

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ILLUSTRATED BY HELIOTYPE AND CHROMO-LITHOGRAPHIC PLATES

AND

TWO HUNDRED AND SIXTY-EIGHT ENGRAVINGS

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among others, by *Bacillus butyricus*. Certain aërobic bacteria also accomplish the same result. Thus Heræus obtained two species from water which reduced nitrates in a very decided manner. On the other hand, a number of species are known to oxidize ammonia, producing nitric acid. Schlösing and Münz, as a result of numerous experiments, arrived at the conclusion that in the soil nitrification is effected by a single species. But it is doubtful whether they worked with pure cultures, and more recent researches show that several, and probably many, different bacteria possess this power. According to Heræus, the following species, tested by him, oxidize ammonia: *Bacillus prodigiosus*, the cheese spirillum of Deneke, the Finkler-Prior spirillum, the typhoid bacillus, the anthrax bacillus, the staphylococci of pus. The oxidation does not always go to the point of forming nitrates, but nitrites may be formed in the soil (Duclaux). Warrington states that certain bacteria which formed nitrates in a suitable culture medium produced only nitrites when, after an interval of four or five months, some of the culture was transferred to a solution containing muriate of ammonia. The same author states that the process of nitrification occurs only in the dark.

The recent researches of Winogradsky, of the Franklands, and of Jordan show that the failure of earlier investigators to obtain the nitrifying bacteria from the soil in pure cultures was due to the fact that these bacteria do not grow in the usual culture media. By the use of certain saline solutions the authors named have succeeded in isolating nitrifying bacteria in pure cultures, or nearly so. It is still uncertain whether these investigators have obtained the same bacteria, but the microorganisms described by them, and obtained from widely distant sources, are similar in their morphological and biological characters, and at least belong to the same group (see Nos. 439, 440, 441). In his latest communication (September, 1891) Winogradsky arrives at the conclusion that the ferments which cause the oxidation of ammonia and production of nitrites are not capable of producing nitrates, but that other microorganisms are concerned in the oxidation of nitrites. In sterilized soil to which a pure culture of his nitromonas was added nitrites only were produced, and the presence of various microorganisms common in the soil did not result in the formation of nitrates so long as the specific ferment was absent to which this second oxidation is ascribed (nitrifying bacillus of Winogradsky, No. 451).

Phosphorescence.—Recently several different bacteria have been studied which, in pure cultures, give rise to phosphorescence in the medium in which they are cultivated. In gelatin cultures the light is sufficient in some instances to enable one to tell the time by a

watch in a perfectly dark room, and such cultures have even been photographed by their own light.

The phosphorescence is influenced by changes in the culture medium and by conditions of temperature, but we have no exact knowledge of the mode of its production. The *Bacillus phosphorescens* from sea water in the vicinity of the West Indies gives the most striking results, especially when planted upon the surface of cooked fish and placed in an incubating oven at 30° C. Two other species have been studied by Fischer—one obtained from the water of the harbor at Kiel, and the other a widely distributed species called by Fischer *Bacterium phosphorescens*. Katz (1891) has recently described several new species obtained by him from sea water and from phosphorescent fish in the markets at Sydney, New South Wales—*Bacillus smaragdino-phosphorescens*, *Bacillus argenteo-phosphorescens*, *Bacillus cyaneo-phosphorescens*, *Bacillus argenteo-phosphorescens liquefaciens* (Nos. 337, 338, 341, and 342).

Morphology.—Straight or slightly curved bacilli with round ends; about two μ long and one-third as broad; grow out into filaments of various lengths.

Biological Characters.—An *aerobic* and *facultative anaerobic*, *liquefying*, *motile* bacillus. Spore formation not observed. Cultures give off a silvery phosphorescence, which is less intense than with the previously described species. Grows at the room temperature in the usual culture media—best at 25° C.; does not grow in the incubating oven at 34° C. Upon *gelatin plates*, at the end of twenty-four hours at the room temperature, small, hyaline discs are developed, which under the microscope are seen to be finely granular and light-brown in color; they are irregularly circular in outline and about 0.7 millimetre in diameter; the deep colonies are considerably smaller, mulberry-like in structure, and straw-yellow in color. At the end of forty-eight hours shallow liquefaction has occurred beneath the superficial colonies, in watch-glass form, and about two millimetres in diameter; under the microscope a central mass of a straw-yellow color is seen, around this a narrow, light-brown zone with granular contents, and outside of this a broader peripheral zone, from which fine, radiating outgrowths are given off into the non-liquefied gelatin. At the same time (forty-eight hours) the deep colonies have a diameter of 0.3 to 0.45 millimetre and a more or less polygonal contour; they are straw-yellow in color and consist of a finely granular central mass, surrounded by a slender, marginal zone which is marked by radial striations. After complete liquefaction of the gelatin the colonies, which remain attached to the glass plate, have a lemon-yellow color. In *gelatin stick cultures* (six per cent) liquefaction occurs beneath the superficial layer which is developed, in form of a shallow watch glass, and gradually extends in diameter and depth; growth also occurs along the line of puncture, and the cultures resemble those of *Bacillus cyano-phosphorescens*, but with less rapid development and liquefaction of the gelatin; also without the formation of hair-like outgrowths into the non-liquefied gelatin. The addition of 2.7 per cent of sodium chloride is favorable for the development of this as for the previously described species of phosphorescent bacilli; on the other hand, the addition of two per cent of glucose exercises a restraining influence upon the growth of all the species studied by Katz. In *bouillon* a diffuse cloudiness is produced by the growth of this bacillus, and a mycoderma is formed upon the surface. No growth occurs in simple meat infusion, but an abundant development when 2.5 per cent of sodium chloride is added to this. Upon *sterilized fish* a shining, sticky, yellowish-gray layer is developed. No growth upon *potato*.

343. BACILLUS PHOSPHORESCENS INDICUS (Fischer).

Found in sea water from the Gulf of Mexico.

Morphology.—Bacilli with rounded and pointed ends, from two to three times as long as broad; length from one-sixth to one-quarter the diameter of a red blood corpuscle; solitary or in pairs; also in short filaments.

Stains readily with the aniline colors, but unstained places are often seen in the interior of the rods.

Biological Characters.—An *aerobic*, *liquefying*, *motile* bacillus. Spore formation not observed. Cultures, especially upon animal substances and in presence of certain soda salts, exhibit a decided phosphorescence in the dark; this depends upon free access of air, and is most marked at a temperature of 25° to 30° C. It is no longer manifested at a temperature of 0° C., and is neutralized by putrefaction. Grows in the usual culture media at the room temperature—not so well in the incubating oven. Upon *gelatin plates*, at the end of thirty-six hours, small, round, grayish-white, punctiform colonies are developed; under a low power these are seen to be spherical, with well-defined outlines, and have a sea-green color with a pink shimmer; later they become granular, and have a wavy outline and a dirty-yellow color. In *gelatin stick cultures*, at the end of four days, a grayish-white line of

growth is seen along the track of the inoculating needle, and at the surface a cup-shaped depression, the size of a hempseed, which contains air; in older cultures the gelatin is liquefied near the surface and a thin, dirty-yellow film swims upon it. Upon the *surface of agar* a grayish-white layer is developed. Upon *potato*, at 15° to 20° C., a thin and broad white layer. Upon *blood serum* a narrow, grayish-white stripe, which extends to a tolerably deep channel, with irregular margins, from 0.5 to 1 centimetre wide; this is lined with a slimy, grayish-white growth. *Cooked fish* or flesh constitutes a favorable medium for the growth of this bacillus. By means of the phosphorescent light given off by cultures of this bacillus, Fischer has succeeded in making photographs not only of the cultures, but of a watch dial placed between two cultures—an exposure of twenty-four hours' duration and a very sensitive dry plate were required to accomplish this.

344. BACILLUS PHOSPHORESCENS INDIGENUS (Fischer).

Found in sea water from the harbor at Kiel and upon phosphorescent herring.

Morphology.—Bacilli with round and slightly pointed ends; somewhat shorter than *Bacillus phosphorescens Indicus*, but of the same thickness—from 1.3 to 2.1 μ long and 0.4 to 0.7 μ broad; solitary or in pairs; may grow out into filaments.

Biological Characters.—An *aerobic*, *liquefying*, *motile* bacillus. Cultures give off a bluish-white, phosphorescent light—not so intense as that from *Bacillus phosphorescens Indicus*; phosphorescence depends upon free access of oxygen. Sea water to which a small amount of a culture is added is phosphorescent in the dark. Spore formation not observed. Grows at the room temperature in the usual culture media—more slowly than *Bacillus phosphorescens Indicus*. Grows at 5° to 10° C., and even below; at a temperature of 32° C. development still occurs, but the cultures do not exhibit phosphorescence. Upon *gelatin plates* the gelatin is depressed about the small spherical colonies, and at the end of a week cylindrical cavities filled with air, and not more than one millimetre in diameter, are formed in the gelatin; at the bottom of these, on the surface of the plate, the colonies are seen; these are the size of a pin's head, thin, disc-formed, and dirty yellow in color; under a low power very young colonies are seen to be circular, with well-defined margins, and of a pale sea-green color; here and there reddish-shimmering granules are seen in the otherwise homogeneous contents; the older colonies are made up of irregular, dirty yellowish-gray masses. In *gelatin stick cultures*, at the end of a week, a conical cavity forms near the surface, which is filled with air and is lined with a thin, friable growth; at the surface the mouth of the cone measures about two millimetres in diameter; this cavity increases in dimensions without containing any liquefied gelatin; in old cultures it may be three to five millimetres in diameter and two to three centimetres deep, the walls being covered with a thin layer of bacilli, and a mass of the same accumulating at the bottom. No growth occurs upon *potato* or upon *blood serum*.

345. BACILLUS CIRCUITANS (Jordan).

Found occasionally in water from the Merrimac River.

Morphology.—Bacilli with round ends, from 2 to 5 μ long and about 1 μ broad; usually solitary, but sometimes in loosely connected chains of three or four elements.

Biological Characters.—An *aerobic* and *facultative anaerobic*, *liquefying*, *motile* bacillus. Forms oval spores, which are located at the ends of the rods and are of about the same diameter as these. Grows in the usual culture media at the room temperature—better at 37° C. Upon *gelatin plates*, at the end of two days, round, brownish colonies become visible; under a low power the liquid contents of these colonies are seen to be in mo-