AN INTERESTING NEW SPECIES OF LUMINOUS BACTERIA

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The purpose of this paper is to give a full description of an interesting new species of luminous bacteria. Its characteristics make it promising material for investigations dealing with the metabolism of such organisms. At the same time it has been thought desirable to give a more complete account than hitherto recorded of another species, extensively studied in the physiological laboratory of Princeton University (Harvey, 1925; Hill, 1932; Shoup, 1929; Taylor, 1934; and others), and identified as *Bacillus Fischeri* (Beij.) Migula (Migula, 1900; Hill and Shoup, 1929), or more recently, according to newer nomenclature, *Achromobacter Fischeri* (Beij.) Bergey *et al.* (Bergey, 1934; Shoup, 1934; Korr, 1935 a, b; Johnson, 1935, a, b). The cultures of *A. Fischeri*, utilized in the present study were originally isolated by Dr. Korr from a dead squid at Woods Hole, Massachusetts, in the summer of 1933. The new species was isolated by the senior author from a dead amphipod (*Talorchestia*, sp.) at Woods Hole, during the summer of 1935. It is morphologically very similar to *A. Fischeri*, but may be readily distinguished from it, as well as from many other species of luminous bacteria, by virtue of the fact that neither its growth nor luminescence is favored by glycerol, and it produces no acid from this substrate in culture. Other definite physiological and cultural characteristics separate it from all adequately described species that have been reported in the literature.[1] Because of the numerous fundamental con-

[1] Molisch (1912) lists as "Halbwegs gekennzeichnet, teilweise gut beschrieben," 30 species described up to that time. Most of these had been studied by Migula

tributions that have been made by E. N. Harvey, not only with respect to the problem of bio-luminescence in general, but also in regard to the luminous bacteria, the name, *Achromobacter Harveyi*, is proposed for this species.

Although neither of these species was isolated directly from sea water, they are almost unquestionably of marine origin. They thrive best in media made up in natural sea water, and will not grow on ordinary media without the addition of salt, the optimum concentration for which is 2.8 to 3.0 per cent, which approximates that in sea water. Furthermore, in common with most marine organisms, they grow best in alkaline media (a fact noted as early as 1894 by Fischer), and are readily injured by acid. For these reasons the organisms were maintained on the $CaCO_3$-buffered sea water medium of Hill (1928) without the glycerol, since with glycerol in the medium enough acid is produced in a few days by *A. Fischeri* to kill the organisms. In determining acid production from carbohydrates, the medium consisted of 0.2 per cent peptone in sea water, with the addition of 2 cc. saturated aqueous solution of phenol red per liter. This broth was tubed in 9 cc. quantities and sterilized in the autoclave. The carbohydrates, with the exception of starch, inulin, and glycogen, which were sterilized with the medium, were made up in a concentration of 10 per cent (or 5 per cent in the case of a few of the rare sugars) in sea water, sterilized by filtration through a Seitz filter, and added in 1 cc. amounts by sterile pipettes to the tubes of broth. These were incubated to prove sterility prior to inoculation. Inoculations consisted of a small loopful of a 24-hour broth culture, or of a dilute sea water suspension of organisms from a 24-hour agar slant.

It has been observed in these studies that when fixed acid is

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(1900). Giard (1889) reported a species of bacteria causing luminescence of various crustacea, including the amphipod *Orchestia*, but from the rather meager descriptions available, was evidently dealing with a different species. In this connection, compare Lehmann and Neumann (1901). For a comprehensive table giving probable synonymies of most of the species of luminous bacteria that were reported in the earlier literature, together with a table giving a synopsis of their characteristics, see Dahlgren (1915).

produced it appears abundantly and unmistakably, whereas non-fixed acid, such as that from CO_2, is more variable. The fixed or non-fixed nature of the acid was determined seven days after inoculation, by aerating the acid cultures with a stream of air. This brings the CO_2 tension of the medium into equilibrium with the atmosphere, and if the acidity has been brought about by an excess of CO_2, the normal pH of approximately 8.0 is quickly restored. With the exception of *A. Harveyi* with salicin, and *A. Fischeri* with glycerol, the production of fixed acid was always sufficient to cause the pH of the cultures to drop from 8.0 to 6.6 (the acid limit of the indicator) within 24 hours at room temperature, accompanied by a diminution or loss of light production. The controls, without added carbohydrate, never showed any indication of acid production beyond a slight, temporary drop in pH of 0.2 at most, during the period of most rapid multiplication of the organisms.

Gas formation from glucose in fermentation tubes could not be detected with either organism.

ACHROMOBACTER HARVEYI, N. SP.

Rods, 0.5 to 1.0 by 1.2 to 2.5 microns, occurring singly or in pairs, with rounded ends (fig. 10). Rods occasionally slightly curved; ends occasionally slightly pointed. Gram-negative. Spores absent. Capsules absent. Motile by means of a single polar flagellum, 2 to 3 times the length of the cell.

Nutrient sea water agar colonies:[2] Mostly very large, reaching in some cases a diameter of 6 to 8 cm. in 24 hours, flat, highly iridescent, circular with more or less undulant borders, or composed of narrow and close or wide filamentous growth. Occasionally small colonies appear that are circular, with entire or slightly undulate edges, often producing irregular secondary growth (figs. 2 to 6), surface always smooth. Luminescent.

Sea water gelatin, pH 7.0. After 24 hours at 20°C., colonies

[2] In many respects similar to colonies figured by Molisch (1904) for *Microspira photogena* Molisch, a species also related to *A. Fischeri*. *A. Harveyi* is most readily distinguished from *M. photogena* by its action on gelatin, since the latter consistently produced no free liquid.

circular, margin slightly undulate, sunken due to the beginning of liquefaction, about 1.5 mm. in diameter or larger, interior somewhat zonate; colonies surrounded by a "halo" of numerous small secondary colonies, circular and finely granular. As a result of rapid liquefaction, 48-hour colonies lose most of their characteristics. In crowded plates, a large number of gas bubbles present. Luminescent.

Sea water agar slant: growth abundant, spreading, greyish, viscous, homogeneous, iridescent, the medium becoming rapidly alkaline when inoculated at an initial pH of 7. With fish decoctions added to the medium, luminescence is much brighter, and growth becomes brownish after several days.

Growth on autoclaved fish: abundant, smooth, glistening, yellowish, becoming a dirty brown after several days. Mild putrefactive odor. Luminescence very brilliant.

Sea water gelatin stab: rapid saccate liquefaction with much flocculent sediment, the gelatin becoming completely liquefied within 5 days at 22°C. (fig. 8).

Potato plugs resting on cotton saturated with sea water: slight growth, somewhat spreading, slightly brownish, luminous.

Milk, with or without the addition of 2.8 per cent NaCl: no growth.

Sea water containing 0.2 per cent peptone: abundant uniform turbidity, thin pellicle, sediment accumulating over a period of several days. Luminescence at surface only unless the tube is shaken.

Indol produced (tested by Goré's method, 1921).

Hydrogen sulphide produced (tested by method of ZoBell and Feltham, 1934).

Ammonia produced in peptone media (tested by method of Hansen, 1930).

Nitrates reduced.

Starch agar: wide zone of hydrolysis of starch.

Fixed acid in: glucose, fructose, mannose, galactose, sucrose, maltose, mannitol, dextrin, glycogen, trehalose, cellobiose; slowly in salicin.

Non-fixed acid: melizitose; slight acid in sorbitol, disappearing after 24 hours.

No acid in: glycerol, xylose, arabinose, dulcitol, inositol, adonitol, erythritol, arabitol, inulin, lactose, raffinose, rhamnose, fucose, alpha methyl glucoside.

Optimum growth temperature: 35° to 39°C., but grows abundantly also at room temperature of 22° to 25°C.

Optimum temperature for luminescence:[3] 20° to 40°C. Luminescence practically absent at 45°; luminescence reduced at 15°, very weak at 10°, very faint at 5°.

Optimum pH for luminescence: 7.4 to 7.8, weaker at 7.0 and 8.2.

Quality of luminescence[4] to completely dark-adapted eyes: yellowish-green to green on fish, typically green on sea water agar or gelatin, with a tendency to become blue-green on agar slants, especially after several days.

Aerobic, facultative anaerobe (proved by growth in phosphate buffered NaCl peptone broth, completely deaerated by a stream of hydrogen purified over hot platinized asbestos and glass-sealed). After growth has taken place anaerobically luminescence occurs only after a period of 1 to 2 hours' aeration.

Not pathogenic when injected into the peritoneum of white rats. Saprophytic on amphipods, but true pathogenicity for these animals not successfully established.

ACHROMOBACTER FISCHERI (BEIJ.) BERGEY ET AL

Rods, 0.4 to 0.8 by 1 to 2.5 microns, occurring singly or in pairs, with rounded ends. Occasional rods slightly curved, ends slightly pointed (fig. 9). Gram negative. Spores and capsules absent. Motile, but organ of motility not successfully determined.

Nutrient sea water agar colonies: small, circular, smooth,

[3] This was determined by subjecting equivalent portions of the same suspension of organisms to different temperatures. The effect of varying the reaction was determined on equivalent portions of a M/2 NaCl suspension added to M/4 phosphate buffer solutions at different pH values.

[4] In describing a luminous species, the "color of light produced" is of doubtful signficance, since it is highly subjective, and could easily have a different meaning to the eyes of the same individual at different stages of dark-adaptation, and furthermore, may vary with the culture medium, age of the culture, or in general, the intensity of the light.

entire, slightly raised, homogeneous, iridescent. Old colonies become yellowish, with margins slightly serrate.

Sea water gelatin colonies: after 48 hours, colonies small (less than 0.5 mm. in diameter) circular, entire, homogeneous, with slight liquefaction.

Sea water agar slant: growth abundant, greyish to yellowish, smooth, viscous, homogeneous, iridescent. With fish decoctions added to the medium, luminescence is no brighter than on nutrient sea water agar with glycerol, if as bright.

Sea water gelatin stab: slight infundibuliform liquefaction, sometimes slightly beaded, tending to become crateriform in old cultures (fig. 7).

Growth on autoclaved fish: moderate, greyish to yellowish, smooth, glistening, luminescent, no odor of putrefaction.

Potato plugs resting on cotton saturated in sea water: growth fairly abundant, spreading, slightly brownish, luminous.

Milk: no growth. Milk plus 2.8 per cent NaCl: slight growth and luminescence but no action on the milk.

Sea water containing 0.2 per cent peptone: growth moderate, mostly near the surface, very thin pellicle; sediment is found in old tubes.

Indol not produced.

Hydrogen sulphide produced.

Ammonia produced in peptone media.

Nitrates reduced.

Hydrolysis of starch doubtful or very slight.

Fixed acid in: glycerol, glucose, fructose, galactose, mannose, maltose, cellobiose,[5] dextrin, salicin.

Non-fixed acid in: mannitol,[5] melizitose.

No acid in: arabinose, xylose, fucose, rhamnose, sucrose, lactose, trehalose, raffinose, glycogen, inulin, adonitol, dulcitol, inositol, sorbitol, erythritol, arabitol, alpha methyl glycoside.

Optimum growth temperature: 25° to 28°C. No growth at 37°C.

[5] In an earlier study by one of us (Johnson, 1935 a, b) evidence was not obtained, by other methods, for the utilization of these substances by washed cells of *A. Fischeri*.

Optimum temperature for luminescence: 28°C. Weak at 10°, none at 5° or at 37°.

Optimum pH for luminescence:[6] 7.4 to 7.8; less intense at 7.0 and 8.2.

Quality of luminescence: typically greenish.

Not pathogenic to white rats.

Aerobic, facultative.

SUMMARY

A complete description is given of a new species of luminous bacteria, designated *Achromobacter Harveyi*, and morphologically quite similar to *Achromobacter Fischeri* (Beij.) Bergey *et al.* A full account of the latter species is also given, in order to complete and summarize earlier data. *Achromobacter Harveyi* is distinguished from *Achromobacter Fischeri* by its carbohydrate fermentations, much more rapid gelatin liquefaction, indol production, more vigorous growth on agar and in broth, large spreading colonies on agar, and high optimum growth temperature.

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[6] This has been studied by Hill (1928).

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PLATE 1

FIG. 1. Typical colony of *A. Fischeri*, 65 hours at room temperature on Hill's sea water agar medium without glycerol. Scale in millimeters. (The lighter crescent zone on the lower edge was caused by refraction of light by the colony.)

FIGS. 2, 3, 4, AND 5. Colonies of *A. Harveyi*, 42 hours at room temperature, on the same medium.

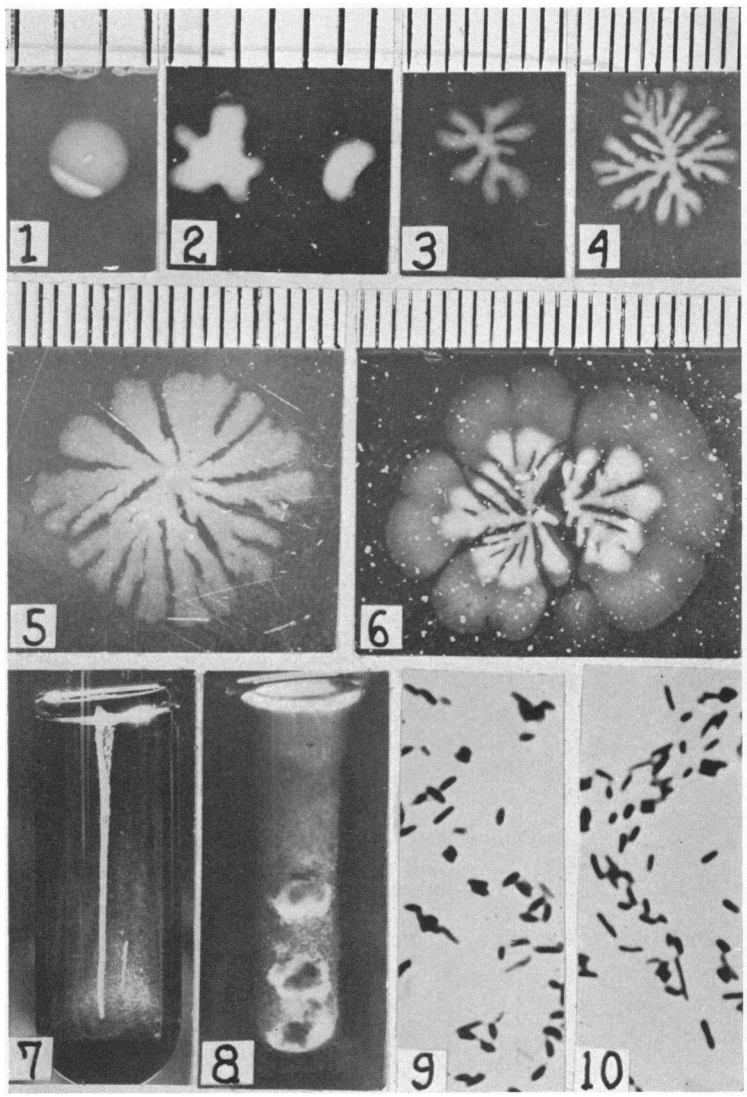
FIG. 6. Colony of *A. Harveyi*, 65 hours old, showing secondary growth. (The small white specks in these photographs are particles of $CaCO_3$ in the medium.)

FIG. 7. *A. Fischeri*, gelatin stab, 4 days at 20° to 22°C.

FIG. 8. *A. Harveyi*, gelatin stab, 4 days at 20° to 22°C.

FIG. 9. *A. Fischeri*, × 2225. From 1-day-old agar slants, stained with crystal violet.

FIG. 10. *A. Harveyi*, × 2225. From 1-day-old agar slants, stained with crystal violet.



(Frank H. Johnson and I. V. Shunk: New Species of Luminous Bacteria)