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The Taxonomy of certain Luminous Bacteria

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SUMMARY: A named strain of each of the species *Photobacterium phosphoreum*, *P. fischeri*, *P. albensis*, *P. pierantonii*, *P. splendidum*, *P. sepia* and *P. harveyi*, together with a recently isolated strain of each of the first two named species, were studied in an attempt to elucidate their correct generic classification. On the basis of morphology, sensitivity to certain antibiotics and a vibriostatic substance, and method of dissimilation of carbohydrates, it was concluded that *P. sepia* and *P. harveyi* should be classified in the genus *Aeromonas*, and the remainder of the species in the genus *Vibrio*.

Although there have been many studies of the mechanism of light production by luminous bacteria (Harvey *et al.* 1948), comparatively few have been devoted to the taxonomy of this group. The work done has been complicated by the fact that these organisms belong to the large group of Gram-negative, non-pathogenic, non-pigmented rods whose differentiation has always been a difficulty. The Dutch workers, following the lead of Beijerinck (1889), classify all luminous bacteria in the genus *Photobacterium* regardless of their differences. This practice has much to commend it from the determinative viewpoint, but it may result in bacteria which belong to different morphological groups being classified together. In *Bergey's Manual* (1948) luminous bacteria are placed in the genera *Pseudomonas*, *Vibrio* and *Bacterium*. As the latter scheme bases the differentiation between *Pseudomonas* and *Vibrio* upon presence or absence of curvature in the bacterial cell, a very variable characteristic, and as the genus *Bacterium* in the *Manual* is an acknowledged collection of unrelated groups, it does not clarify matters. A recent paper by Breed & Lessel (1954) reviewed the literature on the nomenclature of luminous bacteria, but presented no new facts concerning their taxonomy. In recent years, new criteria have been evolved for the differentiation of certain groups of Gram-negative rods, notably the significance of the type of carbohydrate dissimilation (Hugh & Leifson, 1953), and the inhibition by various antibiotics (Shewan, Hodgkiss & Liston, 1954). This paper records the results of applying these and other criteria to a number of strains of luminous bacteria, as part of a general study of the bacterial flora of marine fish, of which luminous bacteria form a not inconsiderable part (Stewart, 1932; Aschehoug & Vesterhus, 1943; Thjøtta & Sømme, 1943; Liston, 1955).

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METHODS

One strain of each of the species *Photobacterium phosphoreum*, *P. sepiæ*, *P. albensis*, *P. pierantonii*, *P. harveyi* and *P. splendidum* were obtained from Professor A. J. Kluyver of Delft. One strain of *P. fischeri* was obtained from Professor J. Cruickshank of Aberdeen University. Strain 2 was isolated from the gut contents of skate and is representative of over fifty almost identical strains obtained from North Sea fish and classified as *P. phosphoreum*. Strain 64 was obtained from the gut contents of mackerel and is classified as *P. fischeri*.

The solid media used consisted of tap-water Lemco agar (Lemco, 10 g.; peptone (Oxoid), 10 g.; NaCl, 5 g., tap water, 1000 ml.; pH 7.6) and sea-water Lemco agar (Lemco, 10 g.; peptone, 10 g.; tap water, 250 ml.; aged sea water, (ZoBell, 1946), 750 ml.; pH 7.6). Tap-water peptone water (peptone, 10 g.; NaCl, 0.5 g.; tap water, 1000 ml.; pH 7.6) or sea-water peptone water (peptone, 10 g.; tap water, 250 ml.; aged sea water, 750 ml.; pH 7.6) were used for liquid media. For the biochemical tests tap-water media alone were used as it was found that these gave good growth in all cases.

For the antibiotic sensitivity tests, tap-water Lemco agar was used as sea water inhibited the action of terramycin to some extent. Tablets containing the indicated amounts of antibiotic were used for penicillin, chloramphenicol, streptomycin and terramycin, and the cup assay method with a saturated aqueous solution for the vibriostatic substance O/129 (2.4-diamino, 6.7-diisopropylpteridine; Shewan *et al.* 1954). The agar plates were surface-poured with a young culture and dried at room temperature for some hours before the antibiotics were applied.

In the determination of the type of dissimilation of carbohydrates, Hugh & Leifson's (1953) medium was used, but with 1.5% NaCl instead of 0.5%. Glucose was the only carbohydrate used as Hugh & Leifson found that organisms which oxidize glucose, but do not ferment it, do not ferment other carbohydrates.

Unless otherwise stated, the temperature of incubation was always at 20°.

Observations on morphology were made on cultures growing on tap-water and sea-water Lemco agar, and in tap-water and sea-water peptone water, at various temperatures, using stained preparations, and also wet preparations by phase-contrast microscopy. Determinations of motility were made on cultures growing in tap-water and in sea-water peptone water, again at various temperatures.

The methods used for the biochemical tests are given in the appropriate column in Table 1. Sugar fermentation and gelatin liquefaction tests were examined up to 28 days of incubation, and the following sugars were used: glucose, lactose, sucrose, mannitol, maltose, starch, glycerol, inositol, rhamnose, xylose, inulin, galactose, glycogen, raffinose, arabinose, sorbitol. The Voges-Proskauer, methyl red, indole production, and nitrate reduction tests were examined after 7 days, and the trimethylamine-oxide reduction test after 9 days of incubation. The trimethylamine-oxide reduction test was used as an

index of possible importance in fish spoilage. Growth temperatures were determined after 72 hr. of incubation in peptone water, with tap water for *Photobacterium albensis* and sea water for the other strains.

RESULTS

The cultural and biochemical characteristics of the strains of luminous bacteria were first determined (Table 1) and compared with previously published characteristics, notably those in *Bergey's Manual* (1948), in Warren's (1945) study of several species of luminous bacteria, in the original description by Johnson & Shunk (1936) of *Photobacterium harveyi*, and a full description of *P. fischeri*. This was necessary to ensure the validity of the named strains and the correct identification of the two unnamed strains.

There appeared to be several differences between the characteristics found in the present study and those previously described. Besides slight anomalies in the production of acid in various sugars, particularly the inability of strain 2, *Photobacterium harveyi* and *P. phosphoreum* to produce acid in sucrose, the main discrepancies were the inability of *P. fischeri* and *P. pierantonii* to liquefy gelatin, of *P. splendidum* to grow at 30 and 37°, and the non-motility of *P. albensis* and *P. pierantonii*. These discrepancies will be discussed later.

The antibiotic sensitivity patterns are shown in Table 2. According to the classification of Shewan *et al.* (1954), *Photobacterium albensis* should be classified as a member of the Achromobacteriaceae because it shows sensitivity to penicillin and to streptomycin. However, the previously described single polar flagellation of this organism and its morphology, vibrio- or spirillum-like, would preclude this classification. Also, as the degree of inhibition is very slight, much less than is generally obtained with tested members of the genus *Achromobacter*, it is considered that *Photobacterium albensis*, like the others, should be placed in the family Pseudomonadaceae. Within this family the species fall into two groups, those sensitive to the vibriostatic compound O/129, and those (*P. sepia* and *P. haveyi*) which are insensitive.

The method of dissimilation of glucose by the various species was in every case fermentative, thus confirming the results of Doudoroff (1942).

The criterion generally used for differentiating the genera *Pseudomonas* and *Vibrio* is that of curvature of the cell, which is present in vibrios and absent from pseudomonads. Consequently, the morphology of the various species was investigated in liquid and on solid media. It immediately became apparent with *Photobacterium phosphoreum* and strain 2 that there were marked discrepancies between previously published descriptions and those now observed. Beijerinck (1889), Stewart (1932), Warren (1945) and *Bergey's Manual* (1948) all emphasized the coccal or coccobacillary morphology of *P. phosphoreum*, but unfortunately none gave the medium used, although Beijerinck did record that they 'prendre dans certaines conditions la forme de spirilles et de vibrios'. Liston (personal communication) has pointed out that although *P. phosphoreum* is coccal or coccobacillary when grown in both tap-water and sea-water peptone water, on tap-water Lemco agar vibrio- and spirillum-like

Table 1. *Characteristics of luminous bacteria*

	Organism							
	Strain 2	<i>P. fischeri</i>	<i>P. sepiæ</i>	<i>P. albensis</i>	<i>P. pier-antonii</i>	<i>P. harveyi</i>	<i>P. splendens</i>	<i>P. phosphoreum</i>
Degree of curvature of the cell	Marked and frequent	Marked and frequent	Occasional and slight	Marked and frequent	Occasional and slight	Very occasional and slight	Marked and frequent	Marked and frequent
Motility	+	+	+	-	-	+	+	+
Flagella (Casare-Gill stain)	Single polar	Single polar	Single polar	-	-	Single polar	Single polar	Single polar
Growth at 0°	+	-	-	-	-	-	-	-
20°	+	+	+	+	+	+	+	+
30°	-	+	+	+	+	+	-	-
37°	-	-	+	+	+	+	-	-
Growth at 20° anaerobically	+	+	+	+	+	+	+	+
Preference for sea-water media	+	+	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+	+	+
Nitrate reduction (Griess-Ilosva,	-	-	+	-	-	+	+	+
Topley & Wilson, 1946)	+	+	+	+	+	+	+	+
Trimethylamine oxide reduction	+	+	+	+	+	+	+	+
(Wood & Baird, 1943)	+	+	+	+	+	+	+	+
Methyl red	-	-	-	-	-	-	-	-
Voges-Proskauer (Barritt, M. M., 1936)	+	-	-	+	-	-	+	+
Indole (Bölune, A., 1906)	-	-	+	-	-	+	-	-
Gas production in sugars	+	+	+	+	+	+	+	+
Acid in the following sugars								
	Glucose	Glucose	Glucose	Glucose	Glucose	Glucose	Glucose	Glucose
	Galactose	Glycerol	Sucrose	Sucrose	Mannitol	Mannitol	Lactose	Sucrose
	Lactose	Mannitol	Maltose	Maltose	Maltose	Maltose	Mannitol	Mannitol
	Maltose	Galactose	Starch	Starch	Starch	Starch	Maltose	Maltose
		Maltose	Galactose	Galactose	Glycerol	Galactose	Starch	Galactose
		Glycerol	Glycerol	Glycerol	Galactose	Glycerol	Glycerol	Glycerol
		Starch	Glycogen	Glycogen	Glycogen	Glycogen	Glycogen	Glycogen

+ = positive; - = negative.

forms are produced. This observation has been confirmed and extended to sea-water Lemco agar (Pl. 1, figs. 1-3). The presence or absence of curvature in the cells of the other species is recorded in Table 1, and typical appearances are shown in Pl. 1, figs. 4-6.

Table 2. *Sensitivity of luminous bacteria to antibiotics*

Organism	Penicillin, 2.5 i.u.†	Chloram- phenicol, 100 µg.†	Strepto- mycin, 80 µg.†	Terramycin, 10 µg.†	Vibriostatic compound O/129*
Strain 2	—	+	+	+	+
<i>P. fischeri</i>	—	+	+	+	+
<i>P. sepia</i>	—	+	+	+	—
<i>P. albensis</i>	±	+	+	+	+
<i>P. pierantonii</i>	—	+	+	+	+
<i>P. harveyi</i>	—	+	+	+	—
<i>P. splendidum</i>	—	+	+	+	+
<i>P. phosphoreum</i>	—	+	+	+	+
Strain 64	—	+	+	+	+

+, sensitive; —, insensitive; ±, slight degree of sensitivity.

* 2-4-diamino, 6-7-di-isopropyl pteridine.

† See p. 112.

DISCUSSION

Certain discrepancies have been noted between characteristics of the luminous bacteria examined, and previous descriptions in the literature. Both *Photobacterium fischeri* and *P. pierantonii* were found not to liquefy gelatin, although the recently isolated strain closely resembling *P. fischeri* does liquefy gelatin. *P. fischeri* was recorded by Johnson & Shunk (1936) and by Warren (1945) as liquefying gelatin only weakly, *Bergey's Manual* (1948) recorded both *Pseudomonas pierantonii* and *Vibrio pierantonii* as not liquefying gelatin, whilst Warren (1945) recorded *Photobacterium pierantonii* as liquefying gelatin. Consequently, the discrepancies found are not thought important.

It is known that the ability to liquefy gelatin is frequently lost on continued subculture on artificial media, as is the property of motility which also appears to have been lost by the strains of *Photobacterium pierantonii* and *P. albensis* studied. The lowering of the maximum growth temperature of *P. splendidum* below 30° may also come into the category of properties lost due to continued subculture in artificial media, particularly as the other characteristics of this species correspond to those previously published. Johnson, Zworykin & Warren (1943) obtained electron micrographs of *P. fischeri* showing lophotrichous flagellation. In the present study this organism was found to possess a single polar flagellum. The difference may merely be due to the different methods of examination.

It thus appears that the strains of luminous bacteria studied are typical members of the named species. Also, although this is not the main purpose of the present work, it appears that the strains of the named species studied have sufficiently distinct characteristics to be regarded as separate species, with the possible exception of *Photobacterium sepia* and *P. harveyi*. Previous work,

notably by Warren (1945), together with the present work, has shown these species to be very similar.

A Gram-negative heterotrophic rod which is motile by one or more polar flagella, is easily classified in the family Pseudomonadaceae. If, however, the rod is non-motile, difficulties are immediately found, but by determining the antibiotic sensitivity pattern of the organisms these difficulties can generally be resolved. By this criterion, the non-motile luminous bacteria studied fall into the Pseudomonadaceae, and the classification of the motile species in this family on the basis of their polar flagellation is confirmed. Within this family the strains can only fall into the genera *Pseudomonas* or *Vibrio*, or possibly the genus *Aeromonas* (Kluyver & van Niel, 1936). On the basis of curvature of the cell, it can be seen (Table 1) that some of the strains fall into the genus *Vibrio*, whilst the remainder, on this criterion alone, should be placed in the genus *Pseudomonas*. All those species showing marked curvature of the cell are also sensitive to the vibriostatic substance, and, as they evince a fermentative dissimilation of glucose, there seems no reason why they should not be placed in the genus *Vibrio*. These species are *Photobacterium fischeri* and strain 64, *P. phosphoreum*, and strain 2, *P. albensis* and *P. splendidum*.

Photobacterium pierantonii shows only slight curvature, but is sensitive to the vibriostatic substance. Many strains of pigmented and non-pigmented pseudomonads, including some *Aeromonas* species, have been examined, and have all been found to be insensitive to the vibriostatic substance (Spencer & Hodgkiss, unpublished). Consequently, it is considered that despite its lack of marked curvature, *P. pierantonii* should be classified as a vibrio.

The two remaining species, *Photobacterium sepia* and *P. harveyi*, due to their lack of curvature and resistance to the vibriostatic compound, cannot be placed in the genus *Vibrio* although they do dissimilate glucose fermentatively. Kluyver & van Niel (1936) suggested that pseudomonads possessing a fermentative metabolism should be separated from those which possess an oxidative metabolism similar to that of *Pseudomonas aeruginosa*, the type species. For the group of fermentative pseudomonads they proposed the generic name *Aeromonas*, a suggestion which has been repeated by several subsequent workers. *Photobacterium sepia* and *P. harveyi* should fall into this genus, except that as originally defined by Kluyver & van Niel, members of the genus should ferment carbohydrates with the production of carbon dioxide and hydrogen. Strains of non-luminous *Aeromonas* studied by the author have this property, but neither *Photobacterium sepia* nor *P. harveyi* produces gas from carbohydrates, or does so only in very small amounts (Doudoroff, 1942). Despite this, it is considered that these two species should be placed in the genus *Aeromonas*, the definition of which should be amended so as not to exclude anaerogenic fermentative pseudomonads. This would be preferable to creating a new genus on the present insufficient knowledge of the metabolism of pseudomonads.

As luminous bacteria have been shown to fall into two distinct genera, indeed, into two distinct tribes in the generally accepted taxonomic scheme, i.e. Pseudomonadeae and Spirillae, it is thought unwise to group them

together in a common genus *Photobacterium*. The suggested generic classification of the luminous bacteria is that the species *phosphoreum*, *albensis*, *pierantonii*, *splendidum* and *fischeri* be placed in the genus *Vibrio*, whilst *sepia* and *harveyi* be placed in the genus *Aeromonas*. This classification is substantially the same as that proposed by Breed & Lessel (1954) who, however, proposed that luminous bacteria of coccoid or coccobacillary form should be placed in a separate genus, *Photobacterium*. As *P. phosphoreum* has been shown to be a curved rod on solid media, and, moreover, sensitive to the vibriostatic agent, this separation is considered unnecessary.

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EXPLANATION OF PLATE

- Fig. 1. Strain 2, tap-water peptone water culture, 18 hr. at 20°, showing coccal and coccobacillary forms. $\times 768$.
- Fig. 2. Strain 2, sea-water Lemco agar culture, 24 hr. at 20°, showing curved rods. $\times 768$.
- Fig. 3. *P. phosphoreum*, sea-water Lemco agar culture, 24 hr., at 20°, showing markedly curved forms. $\times 960$.
- Fig. 4. *P. splendidum*, sea-water peptone water culture, 72 hr. at 20°, showing curved rods. $\times 768$.
- Fig. 5. *P. albensis*, tap-water peptone water, 18 hr. at 20°, showing markedly curved forms. $\times 768$.
- Fig. 6. *P. sepia*, sea-water Lemco agar, 48 hr. at 20°, showing typical straight rods. $\times 768$.

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R. SPENCER—TAXONOMY OF LUMINOUS BACTERIA. PLATE 1

(Facing p. 118)