The Identification, Taxonomy and Classification of Luminous Bacteria

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SUMMARY

The results of a study of 51 strains of luminous bacteria and 12 strains of non-luminous bacteria from related genera show that the luminous bacteria can be divided into three major groups. These groups span three genera, namely *Vibrio*, *Photobacterium* and a suggested new genus, *Lucibacterium*, to accommodate the '*Photobacterium harveyi*' organisms. Revised descriptions of the species are given.

INTRODUCTION

Luminous bacteria are not rare in the marine environment and they have occasionally been reported from non-marine sources. In the sea they are found in the water, as saprophytes and parasites on marine animals and living symbiotically in specialized organs in certain fish and cephalopods. Early workers assigned these bacteria to a variety of genera, but Beijerinck (1889) proposed that all luminous bacteria should be placed in one genus, *Photobacterium*, which is based on a single criterion, namely the ability to emit visible light. Later (1916) Beijerinck stated that some species were closely allied to the cholera vibrios, but since then there has been much confusion in the nomenclature and taxonomy of the luminous bacteria. Many species have been named on ecological grounds, e.g. *Micrococcus physiculus*, *Micrococcus* (*Coccobacillus*) *acropomae*, *Achromobacter merluccius*, *Photobacterium sepiæ*, etc., and some new species have been described which show only minor differences from existing species.

Much of the literature on luminous bacteria deals with the metabolic systems and biochemical requirements of individual strains, but little has been published on the taxonomy of the group as a whole (see McElroy, 1961, for a review). In 1954, Breed & Lessel pointed out that these bacteria had been assigned to no fewer than 15 genera and numerous specified epithets had been used. *Index Bergeyana* (1966) lists 39 specific epithets that have been used with *Photobacterium* alone. Breed & Lessel (1954) proposed that all luminous bacteria should be assigned to two genera, *Photobacterium*, which is placed in the family Pseudomonadaceae, and *Vibrio*. Spencer (1955) studied seven named strains and two new isolates and concluded that all luminous bacteria had close affinities with the genera *Vibrio* and *Aeromonas*.

In the seventh edition of *Bergey's Manual* (1957) all luminous bacteria are listed under *Photobacterium* and *Vibrio* as proposed by Breed & Lessel (1954). In *Photobacterium* there are four species, *Photobacterium phosphoreum*, the type species, *P. fischeri*, *P. harveyi* and *P. pierantonii*. These species are fairly well described, but of the four species listed under *Vibrio* – *Vibrio albensis*, *V. pierantonii*, *V. indicus* and

V. luminosus – only *V. albensis*, a non-marine type, has been adequately described. Prévot (1961), in his *Traité de Systématique Bactérienne*, listed the same eight species under the same genera but selected *P. fischeri* as the type species of Photobacterium. Krassil'nikov (1959) listed eight species in Photobacterium with a further eight sub-species included under five of these species, and one species was included in Pseudomonas – *Pseudomonas harveyi*, a species originally placed in Achromobacter by Johnson & Shunk (1936) and in Photobacterium in *Bergey's Manual* (7th edn) and in Prévot's *Traité de Systématique Bactérienne*.

Table 1. *List of organisms used in the study*

NCMB collection accession number	Species (as received)	Strain and/or other collection numbers	Source
Luminous strains			
1	<i>Photobacterium splendidum</i>	4	A. J. Kluyver, Delft, via R. Spencer
2	<i>P. harveyi</i>	—	
7	<i>P. phosphoreum</i>	—	
24	<i>P. sepiæ</i>	—	
25	<i>P. pierantonii</i>	ATCC 14546	
41	<i>P. albensis</i>	ATCC 14547	J. Cruickshank, University of Aberdeen, via R. Spencer
42	<i>P. fischeri</i>		
60	<i>Micrococcus physiculus</i>	MP	T. Iijima, Osaka, Japan, via R. Spencer
61	<i>Coccobacillus macrouri</i>	CM	
62	<i>Photobacterium phosphoreum</i>	TY	
63	<i>Pseudomonas lucifer</i>	PL	
64	—	FK	
65	—	EB	Y. Yasaki, Tokyo, Japan, via R. Spencer
66	<i>Coccobacillus</i> sp.	HARIDASHIEBISU	
67	<i>Micrococcus acropomae</i>	HOTARUJAKO H	
68	<i>Coccobacillus macrouri</i>	TOHJIN	
69	<i>C. macrouri</i>	SOROIHIGE	
70	—	P 336	T. Iijima, via R. Spencer
71	—	OS	
193	<i>Vibrio</i> sp.	1876	J. Liston, T.R.S. (Liston, 1955)
390	—	I ₂	N. K. Velankar, Mandapam, South India, via R. Spencer (deposited, 1958)
391	—	I ₃	
392	—	I ₄	
393	—	I ₆	
394	—	I ₇	
395	<i>Photobacterium phosphoreum</i>		R. Spencer, Humber Laboratory, Hull
840	—	I ₂ B	N. K. Velankar, via R. Spencer (deposited 1961)
841	—	I ₃ (= 391)	
842	—	I ₇ (= 394)	
843	—	7g	
844	—	DLG 1	
			D. L. Georgala, Fishing Industry Research Institute, Rondebosch, South Africa, via R. Spencer
993	—	21-1-12	U. Melchiorri-Santolini, Fiascherino (La Spezia), Italy

Table 1 (cont.)

NCMB collection accession number	Species (as received)	Strain and/or other collection numbers	Source
1061	<i>Vibrio</i> sp.	314	J. I. W. Anderson, Department of Bacteriology, University of Glasgow
1147	<i>Vibrio</i> sp.	368	R. Spencer, Humber Laboratory, Hull
1148	<i>Vibrio</i> sp.	379	
1149	<i>Vibrio</i> sp.	380	
1150	<i>Vibrio</i> sp.	381	
1151	<i>Vibrio</i> sp.	382	
1152	<i>Vibrio</i> sp.	390	
1198	—	I ₃₄	A. N. Bose, Central Institute of Fisheries Technology, Ernakulam, South India
1199	—	I ₅₂	
1200	—	I ₆₂	
1274	<i>Vibrio noctiluca</i>	U-I	H. Weisglass, Zagreb, Yugoslavia (Weisglass & Skreb, 1963; Weisglass & Gavrilovic, 1963)
1275	<i>Photobacterium profundum</i>	R-I	
1276	—	DLG 2	D. L. Georgala, via R. Spencer
1277	—	DLG 3	
1278	—	F 37	R. Spencer, via J. C. Early
1279	—	CB 95	
1280	<i>Photobacterium harveyi</i>	ATCC 14126	ATCC. Originally deposited in ATCC by F. H. Johnson
1281	<i>P. fischeri</i>	ATCC 7744	
1282	<i>P. phosphoreum</i>	ATCC 11040	
Non-luminous strains			
23	<i>Aeromonas formicans</i>	ATCC 13137 NCIB 9235	I. P. Crawford (1954) (Pivnick & Sabina, 1957)
72	<i>A. hydrophila</i>	G 1	
87	<i>A. liquefaciens</i>	L 417	W. Hodgkiss, T.R.S. A. A. Miles, who obtained it from A. J. Kluyver (Miles & Miles, 1951)
129	<i>Pseudomonas fluorescens</i>	NC 5	
130	<i>Pseudomonas</i> sp.	NC 6	
224	<i>Pseudomonas</i> sp.	139	J. Liston, T.R.S.
320	<i>Pseudomonas</i> sp.	4722	D. L. Georgala, T.R.S.
406	<i>P. putida</i>	A 1005	W. Hodgkiss, T.R.S.
407	<i>Vibrio ichthyodermis</i>	PL 1	W. Hodgkiss & J. M. Shewan, T.R.S. (Hodgkiss & Shewan, 1950; Shewan, Hobbs & Hodgkiss, 1960)
1143	<i>V. marinus</i>	PS 207 ATCC 15382	R. R. Colwell (Colwell & Morita, 1964)
1144	<i>V. marinus</i>	MP-1 ATCC 15381	
NCIB 9243	<i>Aeromonas shigelloides</i>	C 27 ATCC 14030	
			W. H. Ewing (Ferguson & Henderson, 1947)

NCMB = National Collection of Marine Bacteria; NCIB = National Collection of Industrial Bacteria, both at Torry Research Station, Aberdeen. ATCC = American Type Culture Collection; T.R.S. = Torry Research Station.

Clearly there is still much confusion in the classification of luminous bacteria and many of the descriptions given in current editions of manuals are inadequate for present-day requirements. With the desire to clarify the situation, we have made a new study of a collection of these organisms.

METHODS

Organisms. Table 1 lists the 51 strains of luminous bacteria and 12 strains of non-luminous bacteria from 'related' genera that were used.

Media. All sea-water media contained natural sea water, aged as recommended by ZoBell (1946) (storage in the dark for a minimum of 3 weeks) and filtered before use and then diluted, 3 parts sea water to 1 part tap or distilled water. Sea-water-yeast peptone (SWYP): yeast extract powder (Oxoid), 0.3 %; peptone (Evans), 0.5 % at pH 7.4. Agar (1.5 %, Difco) was added for the solid medium. Yeast peptone (pH 7.4): yeast extract powder, 0.3 %; peptone, 0.5 %; NaCl, 0.5 % in distilled water (in some tests the NaCl concentration was altered as required). Glycerol calcium carbonate agar: yeast peptone agar with 3 % NaCl, 1 % glycerol and 0.5 % CaCO₃. Plaice medium (the late Professor A. J. Kluyver, personal communication): minced fresh plaice (200 g.) was soaked in 4 l. tap water for 2 h., boiled for 1 h. and filtered. Peptone (0.5 %) and NaCl (3 %) were added, the broth adjusted to pH 7.3, boiled and filtered. For a solid medium 2 % agar was added. Sea-water lemco: Lab-lemco, 1 %; peptone, 0.5 % at pH 7.4.

Stock cultures were maintained at 20° on SWYP agar or sea-water lemco agar except *Aeromonas shigelloides*, which was maintained on yeast peptone agar. All tests on *A. shigelloides* were carried out in media containing 0.5 % NaCl as growth in sea water or 3 % NaCl media was unsatisfactory.

Temperature relationships. All cultures were streaked on SWYP agar plates, 10 strains per plate, and incubated at 1 and 5° (for 21 days); 15, 20 and 25° (7 days) and 30 and 37° (3 days). The plates were examined at intervals during incubation. As all strains except *Vibrio marinus* grew well at temperatures of 15 to 25° and only some grew at either higher or lower temperatures, all subsequent tests were carried out at 20°, *V. marinus* being tested at 15°.

Morphology. Cultures grown in SWYP broth for 24 or 48 h. were examined under a dry phase contrast microscope (× 240) for motility and shape of the living cells. Strains which were not observed to be motile in liquid medium were examined from 24 h. SWYP agar slope culture as it had been noted that motile forms could occasionally be detected from a solid medium and not from a liquid medium. Smears for staining were prepared from a 2 day SWYP agar culture by suspending the bacteria in 2 % NaCl solution to prevent uptake of water by the cells, and fixed with methanol. The smears were then desalted by flooding with water for 5 min. and allowed to dry before Gram-staining by Jensen's modification (Mackie & McCartney, 1960).

Bacteria required for electron microscopy came from 24 or 48 h. cultures grown on SWYP agar, plaice agar or glycerol calcium carbonate agar. Suspensions were prepared by the following methods. (a) Bacteria were harvested from slopes into 5 % ammonium acetate solution and washed three times by centrifugation at low speed (2000g) and then the deposit was suspended in ammonium acetate. A droplet of this suspension was placed on a grid. (b) Bacteria were harvested into 2.5 % NaCl con-

taining 5 % (v/v) formalin and fixed for 30 min. A drop of this suspension was placed on a grid, dried and was washed by floating the grid face down on distilled water for 10 min. (c) Bacteria were fixed in formalin in sodium chloride solution as in (b), washed twice in 5 % ammonium acetate and a suspension in this solution placed on the grids.

Metal-shadowed preparations were made by drying the suspensions on Formvar-coated 200-mesh copper grids and shadowing with gold + palladium (40 + 60) at an angle of 20°. For negatively stained preparations the suspensions were dried on carbon-stabilized Formvar-coated grids and stained with either 1 % phosphotungstic acid (pH 7; Brenner & Horne, 1959) or 1 % ammonium molybdate. After 1 min. the excess fluid was removed by touching the grid with filter paper. Specimens were examined in a Siemen's Elmiskop I with a double condenser system, a condenser aperture of 200 μ m. and an objective aperture of 50 μ m. Micrographs were recorded on Ilford N50 plates.

Colony appearance was recorded after 3 days incubation on SWYP agar and appearance of growth in liquid medium after 2 days in SWYP broth.

Effect of NaCl on luminescence. Agar slopes of SWYP, plaice medium and glycerol-calcium carbonate media with 0.5 and 3 % NaCl and yeast peptone medium containing different amounts of NaCl (0.5, 1, 1.5, 2, 2.5 and 3 %), were inoculated with a drop of a 2 day broth culture and examined in the dark room daily for 4 days and again after 7 days incubation. The observer spent 10 min. in the dark room before examining the cultures to allow the eyes to become accommodated to the dark.

NaCl requirement and tolerance. Yeast peptone broth with no NaCl and with 5, 7.5 and 10 % NaCl was inoculated from a 2 day SWYP broth culture. The extent of visible growth was recorded after 4, 7 and 15 days incubation, and the tubes were examined for luminescence daily for 4 days.

Antibiotic sensitivity. This was tested by the method of Shewan, Hodgkiss & Liston (1954). The vibriostatic compound '0/129' (2,4-diamino-6,7-diisopropyl pteridine) was applied as filter-paper discs which had been saturated with a 0.1 % solution in dioxan and dried (Schubert, 1962, modified). Yeast peptone agar was used as Spencer (1955) had suggested that sea water interfered with the action of terramycin. Since many strains did not grow enough on this medium to give a satisfactory result, the test was repeated on SWYP agar with compound 0/129 applied in duplicate, as a saturated aqueous solution in cups out from the agar, and as impregnated paper discs.

Biochemical tests. Acid production from carbohydrates and alcohols was tested using 1 % of the substrate and 1 % Andrade indicator in a sea-water peptone medium (1 % peptone in 3:1 sea water:distilled water). The 28 substrates tested were arabinose, rhamnose, ribose, xylose, fucose, glucose, fructose, galactose, mannose, lactose, sucrose, maltose, cellobiose, raffinose, trehalose, sorbose, cellulose, mannitol, dulcitol, sorbitol, glycerol, inositol, salicin, inulin, dextrin, laminarin, starch and glycogen. Results were recorded at 1, 2, 4 and 7 days and then weekly up to 4 weeks.

Dissimilation of glucose was tested by the methods of Hugh & Leifson (1953) and Leifson (1963), but in the latter medium, with filtered aged natural sea water instead of artificial sea water. Results were read after 1, 2, 4 and 7 days, and at 10 and 14 days for strains showing little change after 7 days.

Oxidase was tested by the method of Kovacs (1956).

Tests for indole production, ammonia production from peptone, nitrate reduction,

acetylmethylcarbinol production, methyl red reaction (*Manual of Microbiological Methods*, 1957), 2,3-butanediol production (Bullock, 1961) and trimethylamine oxide (TMO) reduction (Wood & Baird, 1943) were made on 7 day cultures in sea-water media (indole, ammonia, nitrate) or in media supplemented with 3 % NaCl.

Hydrolysis of Tweens was tested by Sierra's method (1957) using media with 3 % NaCl and read at 1, 2, 4 and 7 days.

Møller's method (1955) was used for the detection of lysine and ornithine decarboxylases and the production of alkaline products from arginine. The medium contained 3 % NaCl and results read at 1, 2, 4, 7 and 9 days.

Gelatin liquefaction was tested in stab cultures in SWYP + 12 % gelatin; growth and changes were recorded at 1, 2, 4 and 7 days and weekly to 5 weeks. Strains showing slow liquefaction or no liquefaction were retained for 7 weeks.

Egg yolk reactions were tested on SWYP agar + egg yolk emulsion (Willis & Hobbs, 1958); after 4 days plates were examined and tested for free fatty acids.

Esterase patterns were examined by starch gel electrophoresis by the method of Cann & Willox (1965).

Base compositions of deoxyribonucleic acid were determined by Professor M. Mandel by the buoyant density in caesium chloride method (Mandel, 1966) for 13 luminous strains representative of all the groups and subgroups differentiated by the morphological and biochemical tests. The base composition of the *Pseudomonas* species and three named luminous strains were determined by the thermal denaturation method (Marmur & Doty, 1962) after preparation of the DNA by Marmur's method (1961).

RESULTS

Luminous strains

All luminous strains were Gram-negative rods, and all except three were motile. Gram-stained preparations frequently showed pleomorphic forms. Morphology was most clearly seen in wet preparations under phase contrast and in electron microscope preparations. There were three morphological types.

1. Coccobacilli which, though motile, had a low proportion of motile forms in the culture and frequently appeared non-flagellate. The flagellate forms possessed only a single polar flagellum. Typical of this morphological group is *Photobacterium phosphoreum* (Pl. 1, fig. 1).

2. Motile slender slightly curved rods with a single polar flagellum (strains labelled *Photobacterium splendidum* and *Vibrio albensis*; Pl. 1, fig. 2) or a tuft of polar flagella (strains of *P. fischeri*; Pl. 1, fig. 3).

3. Rods which are often long and unusual in that they have peritrichous flagella, but in addition to the many fine lateral flagella, have a much thicker flagellum at one or both poles. This type of flagellation was seen, for example, in strains of *Photobacterium harveyi* (Pl. 1, fig. 4). Two types of flagella were also seen by Johnson, Zworykin & Warren (1943) in electron microscopic studies of this species, but they did not report the position of attachment.

Preparations of *Photobacterium harveyi* NCMB 1280 showed fascicles of finer flagella (Pl. 2, fig. 5). The difference in the diameters of the thin and thick flagella can be seen in shadowed preparations (Pl. 2, fig. 6). The diameters of the fine flagella were 13 to 18 nm. and those of the thick flagella 30 to 41.6 nm. In negatively stained preparations

the thick flagellum was seen to possess a distinct sheath which was much less stable than the central core (10 to 12 nm. diameter) of the flagellum (Pl. 2, fig. 7, 8). This sheathed structure of the flagellum has been reported for various *Vibrio* species, *Vibrio metschnikovii* (Follett & Gordon, 1963), *V. cholerae* and *V. parahaemolyticus* (Ogasawara & Kuno, 1964*a, b*). Another morphological feature seen in strains of *P. harveyi* was the presence of fimbriae.

Strains of *Photobacterium fischeri* and *Vibrio noctiluca* produced pale yellow pigmented colonies; all others were non-pigmented. No strains produced diffusible pigments.

Only one strain – *Vibrio albensis* – grew in salt-free broth and none grew in 10 % NaCl. Most strains grew in 5 % NaCl and growth in 7.5 % NaCl was confined to strains of *Photobacterium harveyi* and *P. splendidum*. Luminous strains were observed to emit light in media with NaCl concentrations from 0.5 to 3 %, and many were also luminous in yeast peptone broth containing 5 % NaCl. All strains were capable of anaerobic growth.

All strains were insensitive to penicillin, but sensitive to streptomycin, chloramphenicol and polymyxin B; most were sensitive to terramycin. There did not appear to be any definite pattern in the strains insensitive to terramycin. Strains of *Photobacterium harveyi* and *P. splendidum* were insensitive to the vibriostatic compound O/129: all other strains showed some degree of sensitivity to this compound.

Glucose was metabolized fermentatively by all strains in the Leifson (1963) marine OF medium. In the Hugh & Leifson (1953) medium 24 strains were fermentative: the remaining 27 strains did not grow satisfactorily in this medium.

Nitrate was reduced to nitrite by 49 of the 51 strains. The methyl red reaction was positive in 50 strains, four giving a weak result. Ammonia was produced from peptone by all 51 strains. Trimethylamine oxide was reduced by 41 strains, three were negative, the remaining seven strains were not tested.

Of the 28 'sugars' tested, no acid or gas was produced from nine of these; namely, arabinose, rhamnose, xylose, sorbose, raffinose, cellulose, dulcitol, inositol, inulin. A further three sugars were occasionally attacked: ribose (by 12 strains), lactose (by two strains) and fucose (by one strain).

The results of the other tests are summarized in Table 2.

Esterases were seldom detected in strains falling into groups I and II (Table 2) except group Id; but they were detected in strains in groups III and IV. The pattern of esterases within a group was not homogeneous.

Non-luminous strains

The non-luminous strains were all motile Gram-negative rods possessing polar flagella. The *Aeromonas* and *Vibrio* species metabolized glucose fermentatively, were methyl red-positive (except *Aeromonas shigelloides*) and reduced nitrate to nitrite. The *Pseudomonas* species either attacked glucose by the oxidative pathway (NCMB 129, 320, 406) or did not attack glucose (NCMB 130, 224), were negative in the methyl red test and variable in reduction of nitrate. All strains produced ammonia from peptone. The results of the other tests are given in Table 2.

Table 2 (cont.)

Morphology	Hydrolysis of				Egg yolk agar			Møller medium			Growth at 5°	Growth at 37°	Esterases detected	No. of strains	DNA base ratio of representative strain(s)	Species included	Group
	Tween 20	Tween 40	Tween 60	Tween 80	Opalescence	Free fatty acid	Arginine	Lysine	Ornithine	Compound o/129 sensitivity							
Coccobacilli, motile with a single polar flagellum, or non-motile	+	+	-	-	-	-	+	+	-	+	+	-	(1+)	5	42.0	<i>P. phosphoreum</i>	Ia
	-	-	-	-	-	-	+	+	-	+ or ±	+	-	(1+)	13	40.5	<i>P. profundum</i>	Ib
	(2+)	(2+)	-	-	-	-	(1-)	+	(1+)	-	+	-	(1+)		40.5	<i>C. macroauri</i>	
																<i>M. acropomae</i>	
Rods, motile with a tuft of polar flagella	-	-	-	-	-	-	+	+	-	+	+	-	-	3	39.5	<i>Ps. physcillus</i>	Ic
	+	+	+	+	+	+	+	+	-	±	+	+	+	4	42.0	<i>Ps. lucifer</i>	Id
	+	+	+	+	+	+	-	(1-)	-	+	-	-	-	1	39.0	<i>P. pierantonii</i>	IId
	+	+	+	+	+	+	-	+	+	+	+	+	+	10	43.5	<i>P. fischeri</i>	IIa
Rods, motile with 1 to 4 polar flagella	+	+	+	+	+	(1+)	-	+	+	+	(2-)	(2+)	+	1	45.5	<i>V. noctiluca</i>	IV
	+	+	+	+	+	-	-	+	+	+	-	-	+	1	48.5	<i>V. albensis</i>	IV
	+	+	+	+	+	+	+	+	+	+	+	+	+	1	45.0	<i>P. splendidum</i>	IIIb
	+	+	+	+	+	+	-	+	+	-	-	+	+	13	46.0	<i>P. harveyi</i>	IIIa
Rods, motile with peritrichous flagella	+	+	+	+	+	+	(1+)	+	(1-)	-	-	-	(2-)		46.0	<i>P. sepiac</i>	
	+	+	+	+	+	+	+	-	-	+	+	-	+	1	44.5 ¹	<i>V. ichthyoderms</i>	
	+	+	+	+	+	+	-	+	-	+	+	-	+	2	40.0 ²	<i>V. marinus</i> ⁴	
	+	+	+	+	+	+	-	+	-	+	+	-	+		42.0 ²		
Rods, motile with many polar flagella	+	+	+	+	n.t.	n.t.	+	+	+	+	-	+	+	1	51.0 ³	<i>A. shigelloides</i>	
	+	+	+	+	-	-	+	+	-	-	-	+	+	1	59.0 ³	<i>A. formicans</i>	
	+	+	+	+	-	-	+	+	-	-	+	+	+	2	59.0 ²	<i>A. liquefaciens</i>	
	+	+	+	+	-	-	+	+	-	-	+	+	+		62.0	<i>A. hydrophila</i>	
Rods, motile with 1 to 8 polar flagella	+	+	-	-	+	+	+	-	-	-	+	-	+	1	64.2	<i>Ps. fluorescens</i>	
															Gp I ³		
	+	+	-	-	-	-	+	-	-	-	+	-	+	1	63.2	<i>Ps. putida</i> Gp I	
	+	+	-	-	-	-	+	-	-	-	+	-	+	1	60.8	<i>Pseudomonas</i> sp. Gp II	
Rods, motile with 1 to 4 polar flagella	+	+	+	+	-	-	-	-	-	-	+	-	+	2	42.2	<i>Pseudomonas</i> sp. Gp III ³ IV ³	
															41.9		

A, acid; a, acid weak and late; G, gas; +, positive; ++, strong or rapid positive; ±, weak or doubtful positive; n.t., not tested; ¹, results of Véron & Sebald, 1964; ², from Hill, 1966; ³, the *Pseudomonas* groupings of Shewan, Hobbs & Hodgkiss, 1960; ⁴, one strain of *V. marinus* (NCMB 1144) did not grow in some of the Tween 80 and Møller media. Figures in parenthesis indicate the number of strains giving a different result.

DISCUSSION

Table 2 shows that 44 of the luminous strains fall into three major groups (Ia, b, c, IIa, IIIa) which correspond well with the three morphological divisions. Of the remaining seven strains, *Vibrio albensis*, the only non-marine strain, is unique, whereas the other six strains appear to be transitional between the main morphological and biochemical types, particularly the two strains labelled *Photobacterium pierantonii* and *P. splendidum*.

Groups Ia, b and c are all very similar and such differences as do exist appear to be of minor importance and to represent variation of strains within a single species, e.g. group Ib produces acid and gas from carbohydrates whereas group Ic is anaerogenic, otherwise they are identical. The only differences between group Ia and groups Ib and c are the variability in the VP and 2,3-butanediol tests in Ia and their ability to hydrolyse Tweens 20 and 40, but two strains in Ib also hydrolyse these Tweens. All group I strains examined have DNA base ratios of 39.5 to 42.0 moles % guanine + cytosine (% G + C). We recommend that all 21 strains in groups Ia, b and c should be combined in the species *Photobacterium phosphoreum*. Spencer (1955) proposed that *P. phosphoreum* should be assigned to the genus *Vibrio* as it was sensitive to the vibriostatic compound O/129 and dissimilated glucose fermentatively, but all the strains in this group (*P. phosphoreum*) are Kovacs' oxidase-negative and are usually aerogenic in the breakdown of carbohydrates whereas *Vibrio* species are oxidase-positive and never aerogenic. The species *P. phosphoreum* should include species previously labelled *P. profundum* (Weisglass & Gavrilovic, 1963), *Pseudomonas lucifer*, *Coccobacillus macrouri*, *Micrococcus physiculus* and *Micrococcus* (or *Coccobacillus*) *acropomae*.

The position of group Id is further away from groups Ia, b and c than these are from each other. Although the group Id strains have much in common with the other group I strains, they attack hexoses only, are Kovacs' oxidase-positive, hydrolyse Tweens 20 and 40 (some strains also Tweens 60 and 80) and although VP-positive, they are 2,3-butanediol negative. The groups Ia, b and c strains attack maltose in addition to hexoses, are oxidase-negative and never hydrolyse Tweens 60 and 80. The DNA base ratio of the one strain from Id examined was identical with a strain of *Photobacterium phosphoreum* in group Ia (42.0 moles % G + C). This group appears to form a separate species, and we propose the name *P. mandapamensis*, since the first strain of this group was isolated at Mandapam.

The 10 strains in group IIa fit closely to the second morphological type and are different from the organisms of group I in being oxidase-positive, not giving an alkaline reaction with arginine, and attacking a much wider range of carbohydrates anaerogenically. They are also the only pigmented group, the colonies being pale yellow. The DNA base ratios of the strains examined are 43.5 and 45.5 moles % G + C. All strains in this group should be combined under the specific epithet *fischeri* which should be assigned to the genus *Vibrio* as suggested by Spencer (1955) as they are polar flagellate rods, fermentative in their action upon glucose, sensitive to compound O/129 and also have a DNA base ratio within the range for that genus (40 to 50 moles % G + C; Colwell & Mandel, 1964). Furthermore, they are very similar to the working neotype strains of the non-luminous species *Vibrio marinus* (Colwell & Morita, 1964). Included in the species *V. fischeri* is the recently described species *V. noctiluca* (Weisglass & Skreb, 1963).

The 13 strains in group IIIa also form a single species which differs from group I

and group IIa in being insensitive to the vibriostatic compound o/129, indole-positive, much more actively proteolytic in gelatine, having an ornithine decarboxylase (except *Photobacterium harveyi* NCMB 1280, the type strain) and lipases which are active in egg yolk agar, in addition to having the peritrichous flagella of the third morphological type. The DNA base ratios of the strains examined were 45.5 to 46.5 moles % G + C. The strains in this group should be combined under the specific epithet *harveyi* and should include the strain labelled *P. sepiac*, a name which is not considered to be legitimate (*Index Bergeyana*, 1966), and NCMB 42 which was originally labelled *P. fischeri*. Spencer (1955) believed that this group had affinities with the genus *Aeromonas* as it was insensitive to compound o/129. However, the *harveyi* group is peritrichate and has a DNA base ratio (45.5 to 46.5 moles % G + C) which is well outside the range of the genus *Aeromonas* (59 to 62 moles % G + C) (Hill, 1966; Rosypal & Rosypalova, 1966). We recognize that this group (IIIa) has some affinities with the genus *Vibrio*, having a similar DNA base ratio and the sheathed structure of the thick polar flagellum seen in some strains is similar to that described for some *Vibrio* species (Follett & Gordon, 1963; Ogasawara & Kuno, 1964*a, b*). However, the possession of peritrichous flagella and insensitivity to compound o/129 leads us to suggest that the species should be assigned to a new genus for which we propose the name *Lucibacterium* (Latin: *lux*, *lucis*, light) for the genus with *Lucibacterium harveyi* (Johnson & Shunk) comb.nov. as the type species.

The strain labelled *Photobacterium splendidum* (group IIIb) is identical with group IIIa except that it has a single polar flagellum and a lower temperature range. This strain has been maintained in artificial culture for many years and it is possible that some of its properties may have been irretrievably lost, as has happened with some strains of the *Vibrio fischeri* group which are no longer luminous, although this property was observed by us when they were freshly isolated. The DNA base ratio of *P. splendidum* (45.0 moles % G + C) is also near that of the group IIIa strains and we believe that this organism may be a degenerate *Lucibacterium harveyi*.

The organism labelled *Photobacterium pierantonii* (group IIb) is morphologically similar to those in group I, being a non-motile coccobacillus and having a low DNA base ratio (39.0 moles % G + C) similar to other strains in this group. Biochemically this organism is almost identical with those of group IIa. In the seventh edition of *Bergey's Manual* (1957) there are two luminous species described which have the specific epithet *pierantonii* – *Photobacterium pierantonii* and *Vibrio pierantonii*. Our strain does not fit exactly the description of either species. Biochemically it is close to IIa *V. fischeri*, but morphologically is more like the *Photobacterium* species and its temperature range did not reach the value quoted for *V. pierantonii*. This might possibly be a single representative of a species but for the present we propose to group it as an atypical strain of *V. fischeri*.

The non-marine strain *Vibrio albensis* (group IV) is distinct from all the others. Morphologically it is similar to group IIa, being a slender rod with one or more polar flagella. It is similar to group III in being actively proteolytic, indole-positive, but it is the only strain outside group I which is VP and 2,3-butanediol-positive. It has decarboxylases for lysine and ornithine like many other *Vibrio* species (Bain & Shewan, 1968; Carpenter, Hart, Hatfield & Wicks, 1968). The DNA base ratio was the highest of all the strains examined at 48.5 moles % G + C and is close to that of *V. cholerae*. Its similarity to *V. cholerae* has long been recognized (*Bergey's Manual*, 1957).

It is therefore suggested that luminous bacteria should be classified into three genera, namely *Photobacterium*, *Vibrio* and a new genus, *Lucibacterium*, to accommodate organisms currently called *P. harveyi* and *P. sepiæ*. The species to be included in the genus *Vibrio* are *V. albensis* and *V. fischeri*, and in the genus *Photobacterium*, *P. phosphoreum*, the present type species, with the addition *P. mandapamensis* as a further distinct species. The genus *Lucibacterium* would include *P. splendidum* as a degenerate form of *L. harveyi*.

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APPENDIX

Our proposals for (i) modified and extended descriptions of the species named in this appendix and (ii) the generic descriptions of *Photobacterium* and *Lucibacterium*, are based on information from the present study, from the seventh edition of *Bergey's Manual* (1957) and from the work of Spencer (1961) on the chitinoclastic activities of luminous bacteria.

Species included in the genus *Vibrio* Pacini, 1854

Vibrio albensis Lehmann & Neumann, 1896

(Synonyms: Elbe vibrio, Dunbar, 1893; *Microspira dunbari* Migula, 1900.)

Rods, 1.5 to 3.0 × 0.4 to 0.7 μ m., occurring singly and in pairs, sides parallel, ends rounded, axis frequently curved. Motile by means of polar flagella, usually one per cell, but occasional cells may have 2 or 4 flagella. Not encapsulated. Gram-negative.

Agar colonies: Off-white, translucent, circular, convex, entire margin, smooth, shiny, 1.0 to 2.0 mm. diam. Good growth. Luminous. On sea-water agar the colonies are punctiform and luminous.

Broth: Good growth, thin pellicle, uniform turbidity, viscid deposit. Luminous 2 to 3 days.

Grows at 37° but not at 5°. Grows in media containing 0 to 5 % NaCl, no growth in 7.5 % NaCl, luminescent in media with 0.5 to 3 % NaCl. Growth in media at pH 6 to pH 9, no growth at pH 5.

Aerobic, facultatively anaerobic.

Sensitive to chloramphenicol, streptomycin, aureomycin, terramycin, polymyxin B and 2,4-diamino-6,7-diisopropyl pteridine. Insensitive to penicillin.

Indole produced, acetylmethylcarbinol produced, 2,3-butanediol produced, nitrite produced from nitrate, ammonia produced from peptone, trimethylamine oxide reduced to trimethylamine. Methyl red test negative.

Oxidase- and catalase-positive.

Possesses decarboxylases for lysine and ornithine but does not produce alkaline products from arginine.

Hydrolyses Tweens 20, 40, 60 and 80.

No opalescence or free fatty acids produced on egg yolk agar.

Gelatin liquefied.

Starch hydrolysed.

Chitin digested.

Acid but no gas formed in glucose, sucrose, maltose, trehalose; weak acid production in fructose, mannose, mannitol, sorbitol, dextrin, starch and glycogen. No acid or gas in arabinose, rhamnose, xylose, ribose, fucose, galactose, lactose, sorbose, raffinose, cellobiose, cellulose, dulcitol, glycerol, inositol, adonitol, salicin, inulin, aesculin or laminarin.

Carbohydrate metabolism fermentative.

DNA base ratio 48.5 moles % G + C.

Habitat: Fresh water, in human faeces and bile.

Vibrio fischeri (Beijerinck) Lehmann & Neumann, 1896.

(Synonyms: Die einheimischen Leuchtbacillen, Fischer, 1888; *Photobacterium fischeri* Beijerinck, 1889; *Bacillus fischeri* (Beijerinck) Trevisan, 1889; *Bacillus phosphorescens indigenus* Eisenberg, 1892; *Bacterium phosphorescens indigenus* Chester, 1897; *Microspira fischeri* (Beijerinck) Chester, 1901; *Spirillum phosphorescens* Holland, 1920; *Vibrio phosphorescens* Holland, 1920; *Achromobacter fischeri* (Beijerinck) Bergey *et al.* 1930; *Vibrio noctiluca* Weisglass & Skreb, 1963.)

Short rods, 0.5×1.0 to $1.5 \mu\text{m}$, occurring singly and in pairs, sides parallel, ends rounded, axis straight or slightly curved. Stained preparations frequently show pleomorphism and polar staining. Motile by means of a tuft of polar flagella. Gram-negative.

Sea-water agar colonies: Off-white in young cultures, becoming pale yellow, translucent, circular, convex, entire margin, smooth, shiny 1.0 to 2.0 mm. diam. Usually luminous at 1 to 3 days. Growth moderately good.

Sea-water broth: Abundant growth, ring or thin pellicle, uniform turbidity, small deposit, usually luminous at 2 to 3 days.

Usually grows at 5° but not at 37° . Growth and visible light usually emitted in media containing 0.5 to 5 % NaCl, some strains grow in 7.5 % NaCl; no growth without NaCl. Growth from pH 6 to pH 9.

Aerobic, facultatively anaerobic.

Sensitive to chloramphenicol, streptomycin, polymyxin B and 2,4-diamino-6,7-diisopropyl pteridine. Insensitive to penicillin. Sensitivity to terramycin and aureomycin varies from strain to strain.

Nitrite produced from nitrate; trimethylamine oxide reduced to trimethylamine; ammonia produced from peptone. Indole, acetylmethylcarbinol and 2,3-butanediol not produced.

Methyl red test positive.

Oxidase- and catalase-positive.

Usually has a lysine decarboxylase but not an ornithine decarboxylase nor produces alkaline products from arginine.

Tweens 20 and 40 hydrolysed, Tweens 60 and 80 usually hydrolysed.

Opalescence produced in egg yolk agar, production of free fatty acids variable.

Gelatin liquefied.

Starch hydrolysed.

Chitin may be digested.

Acid but no gas formed in glucose, fructose, galactose, mannose, maltose, dextrin and starch, and by some strains in cellobiose, trehalose, mannitol, glycerol, salicin and

glycogen. No acid or gas formed in arabinose, rhamnose, ribose, xylose, fucose, lactose, sucrose, sorbose, raffinose, cellulose, dulcitol, sorbitol, inositol, inulin or laminarin.

Metabolism of carbohydrates fermentative.

DNA base ratio 43 to 45 moles % G + C.

Habitat: Sea water.

Photobacterium Beijerinck, 1889

Coccobacilli or occasional rods, pleomorphic forms frequently observed in adverse conditions of growth, motile by means of polar flagella or non-motile. Many strains appear to be non-motile as only a low proportion of the cells in a culture are seen to move actively and detection of flagella is difficult. Chemo-organotrophic, aerobic and facultatively anaerobic. Acid and gas or acid only produced from glucose and other carbohydrates but not lactose, do not attack complex carbohydrates or alcohols. Carbohydrate metabolism is fermentative. Nitrite produced from nitrate. Usually luminescent. Growth and luminescence occur on media containing 0.5 to 5 % NaCl; optimum concentration 3 % NaCl. Growth at pH 6 to pH 9. Most strains grow at 5 but not at 37°. Usually sensitive to 2,4-diamino-6,7-diisopropyl pteridine. DNA base ratio 39 to 42 moles % G + C. Found living symbiotically in the tissues of luminous organs of cephalopods and deep-sea fishes and on the skin and in the intestine of some marine fish.

The type species is *Photobacterium phosphoreum* (Cohn) Ford.

Key to the species of the genus *Photobacterium*:

Coccobacilli which produce acid and usually gas from glucose.

A. Oxidase-negative, acid and usually gas from maltose, no growth at 37°. *Photobacterium phosphoreum*.

B. Oxidase-positive, no acid or gas from maltose, growth at 37°. *Photobacterium mandapamensis*.

Photobacterium phosphoreum (Cohn) Ford, 1927

(Synonyms: *Micrococcus phosphoreus* Cohn, 1878; *Bacillus phosphorescens* II Baumgarten, 1888; *Photobacterium phosphorescens* Beijerinck, 1889; *Bacillus hermesii* Trevisan, 1889; *Streptococcus phosphoreus* (Cohn) Trevisan, 1889; *Bacillus phosphoreus* (Cohn) Macé, 1901; *Photobacter phosphorescens* Beijerinck, 1901; *Pseudomonas lucifera* Molisch, 1904; *Bacterium phosphoreum* (Cohn) Molisch, 1912; *Photobacter phosphoreum* (Cohn) Beijerinck, 1916; *Micrococcus physicululus* Kishitani, 1930; *Coccobacillus acropoma* Yasaki & Haneda, 1936; *Coccobacillus macrouri*; *Acinetobacter phosphorescens* Brisou, 1955; *Photobacterium profundum* Weisglass & Gavrilovic, 1963.)

Short rods or coccobacilli 1.0 to 2.5 × 0.4 to 1.0 µm., occurring singly and occasionally in pairs, axis straight. Motile by a single polar flagellum, but only a small proportion of the cells in a culture are observed in motion. In stained specimens many pleomorphic cells are observed and polar staining frequently occurs. Gram-negative.

Sea-water agar colonies: Off-white, translucent, circular, convex, entire margin, smooth, shiny, 1 to 2 mm. diameter. Luminous 1 to 3 days. Moderately good growth.

Sea-water broth: Moderately good growth, ring of dense growth at surface, slight uniform turbidity, granular deposit.

No growth at 37°, most strains grow at 5°. Growth and visible light usually emitted in media containing 0.5 to 5 % NaCl. No growth in 7.5 % NaCl or without NaCl. Growth at pH 6 to pH 9.

Aerobic, facultatively anaerobic.

Sensitive to chloramphenicol, streptomycin, polymyxin B and 2,4-diamino-6,7-diisopropyl pteridine; sensitivity to terramycin and aureomycin is variable. Insensitive to penicillin.

Nitrite produced from nitrate, acetylmethylcarbinol and 2,3-butanediol produced, ammonia produced from peptone, trimethylamine oxide reduced to trimethylamine. Indole not produced.

Methyl red test positive.

Oxidase-negative, catalase-positive.

Usually produces alkaline products from arginine and possesses a lysine decarboxylase but does not possess an ornithine decarboxylase.

Tweens 20 and 40 occasionally hydrolysed, Tweens 60 and 80 not hydrolysed.

No opalescence or free fatty acids produced on egg yolk agar.

Gelatin not usually liquefied.

Starch not hydrolysed.

Chitin usually digested.

Acid and usually gas formed in glucose, fructose, galactose, mannose and maltose and occasionally in ribose and fucose. No acid or gas formed in arabinose, rhamnose, xylose, lactose, sucrose, sorbose, cellobiose, trehalose, raffinose, cellulose, mannitol, dulcitol, glycerol, sorbitol, inositol, dextrin, salicin, inulin, laminarin, starch or glycogen.

Metabolism of carbohydrates fermentative.

DNA base ratio 39.0 to 42.0 moles % G + C.

Habitat: Sea water.

Photobacterium mandapamensis sp.nov.

Short rods or coccobacilli 1.0 to 2.5 × 0.4 to 1.0 μm., occurring singly and occasionally in pairs, axis straight. Motile by polar flagella. In stained specimens pleomorphic cells and polar staining may be observed. Gram-negative.

Sea-water agar colonies: Off-white, translucent, circular, convex, entire margin, smooth, shiny 1 to 2 mm. diameter. Luminous 1 to 3 days. Moderately good growth.

Sea-water broth: Moderately good growth, thin pellicle, uniform turbidity, powdery deposit. Luminous 2 to 3 days.

Grows at 37° but not at 5°. Growth and visible light usually emitted in media containing 0.5 to 5 % NaCl. No growth without NaCl or in 7.5 % NaCl. Growth at pH 6 to pH 9.

Aerobic, facultatively anaerobic.

Sensitive to chloramphenicol, streptomycin, polymyxin B and 2,4-diamino-6,7-diisopropyl pteridine. Sensitivity to terramycin and aureomycin is variable. Insensitive to penicillin.

Nitrite produced from nitrate, trimethylamine oxide reduced to trimethylamine, ammonia produced from peptone, acetylmethylcarbinol produced but 2,3-butanediol not produced. Indole not produced.

Methyl red test positive.

Oxidase- and catalase-positive.

Usually produces alkaline products from arginine and possesses a lysine decarboxylase, but not an ornithine decarboxylase.

Tweens 20 and 40 hydrolysed, Tweens 60 and 80 may be hydrolysed.

No opalescence or free fatty acid produced on egg yolk agar.

Gelatin may be slowly liquefied.

Starch not hydrolysed.

Chitin may be digested.

Acid and gas formed in glucose, fructose, galactose and mannose. No acid or gas formed in arabinose, rhamnose, ribose, xylose, fucose, lactose, sucrose, maltose, trehalose, sorbose, cellobiose, raffinose, cellulose, mannitol, dulcitol, sorbitol, glycerol, inositol, salicin, dextrin, inulin, laminarin, starch or glycogen.

Metabolism of carbohydrates fermentative.

DNA base ratio 42.0 moles % G + C.

Habitat: Sea water.

Lucibacterium gen.nov.

Rods, motile by peritrichous flagella. In some strains the flagellation is of an unusual type with a thick polar flagellum in addition to the finer lateral flagella. Occasionally only the polar flagellum can be detected. Chemo-organotrophic. Aerobic, facultatively anaerobic. Carbohydrate metabolism is fermentative without the production of gas. Lactose not usually attacked. Gelatin liquefied. Nitrite produced from nitrate. Oxidase-positive. Insensitive to 2,4-diamino-6,7-diisopropyl pteridine. Usually luminescent. Growth and luminescence occurs on media containing 0.5 to 5 % NaCl, optimum concentration 3 % NaCl. Growth at pH 6 to pH 9. Usually grows at 37° but not at 5°. DNA base ratio 45 to 46 moles % G + C. Found on the surface of dead fish and in sea water.

The type species is *Lucibacterium harveyi* (Johnson & Shunk) comb.nov.

Lucibacterium harveyi (Johnson & Shunk)

(Synonyms: *Achromobacter harveyi* Johnson & Shunk, 1936; *Photobacterium splendidum* (Beijerinck) Eymers & van Schouwenburg, 1937; *Photobacterium sepiiae* Kluyver, 1938; *Photobacterium harveyi* (Johnson & Shunk) Breed & Lessel, 1954; *Pseudomonas harveyi* (Johnson & Shunk) Krassil'nikov, 1959.)

Rods, 1.2 to 4.0 × 0.3 to 1.0 μ m., straight or slightly curved, sides parallel, ends rounded, occurring singly and in pairs. Pleomorphic form may be present. Motile by means of peritrichous flagella. Flagellation may be of an unusual type showing a thick polar flagellum in addition to many fine lateral flagella. In some strains only the polar flagellum can be detected. Gram-negative.

Sea-water agar colonies: Off-white, translucent, circular, convex, entire margin, smooth, shiny, 1 to 3 mm. diameter. Colonies frequently mucoid and tend to spread. Abundant growth. Luminous after 1, 2 or 3 days.

Sea-water broth: Good growth, thin pellicle, uniform turbidity, flocculent deposit. Luminous.

Usually grows at 37° but not at 5°. Grows in media containing 0.5 to 7.5 % NaCl,

but not in media without NaCl. Visible light emitted in media containing 0.5 to 5 % NaCl. Growth at pH 6 to pH 9.

Aerobic, facultative anaerobic.

Sensitive to chloramphenicol, streptomycin, aureomycin, may be sensitive to terramycin. Insensitive to penicillin and 2,4-diamino-6,7-diisopropyl pteridine.

Indole produced, nitrite produced from nitrate, ammonia produced from peptone, trimethylamine oxide reduced to trimethylamine.

Acetylmethylcarbinol and 2,3-butanediol not produced.

Methyl red test positive.

Oxidase- and catalase-positive.

Usually possesses decarboxylases for lysine and ornithine, but does not usually produce alkaline products from arginine.

Tweens 20, 40, 60 and 80 hydrolysed.

Opalescence and free fatty acids produced in egg yolk agar.

Gelatin liquefied.

Starch hydrolysed.

Chitin digested.

Acid but no gas produced from glucose, fructose, galactose, mannose, maltose, cellobiose, trehalose, mannitol, sorbitol, dextrin, laminarin, starch and glycogen. Acid may be produced from ribose, sucrose, glycerol, salicin and occasionally from lactose (weak). No acid or gas from arabinose, rhamnose, xylose, fucose, sorbose, raffinose, cellulose, dulcitol, inositol or inulin.

Metabolism of carbohydrates fermentative.

DNA base ratio 45 to 46 moles % G + C.

Habitat: Sea water.

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EXPLANATION OF PLATES

All the photographs are electron micrographs of preparations from cultures incubated at 20° on SWYP agar for 24 h. and fixed in 5 % (v/v) formalin in 2.5 % NaCl. Fig. 1 to 6 inclusive are of gold + palladium shadowed preparations. Fig. 7 and 8 are of specimens negatively stained with phosphotungstic acid.

PLATE 1

Fig. 1. *Photobacterium phosphoreum* NCMB 7.

Fig. 2. *Lucibacterium harveyi* NCMB 1 (*Photobacterium splendidum*).

Fig. 3. *Vibrio fischeri* NCMB 1281 (*Photobacterium fischeri*).

Fig. 4. *Lucibacterium harveyi* NCMB 1280 (*Photobacterium harveyi*).

PLATE 2

Fig. 5. *Lucibacterium harveyi* NCMB 1280 (*Photobacterium harveyi*). Thick and thin flagella.

Fig. 6. *Lucibacterium harveyi* NCMB 1280 (*Photobacterium harveyi*). Fascicles of thin flagella.

Fig. 7. *Lucibacterium harveyi* NCMB 1280 (*Photobacterium harveyi*). Thick (polar) flagellum showing core and sheath.

Fig. 8. *Lucibacterium harveyi* NCMB 1280 (*Photobacterium harveyi*). Two thick (polar) flagella showing disintegration of the sheath.