Numerical Taxonomy of Violet-Pigmented, Gram-Negative Bacteria and Description of *Iodobacter fluviatile* gen. nov., comb. nov.

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A total of 113 violet chromogens, 45 of which produced spreading colonies characteristic of *Chromobacterium fluviatile*, were isolated from fresh water. These isolates and 27 other chromobacteria, 9 duplicates, and 11 reference strains were subjected to 95 characterization tests, and similarities were computed by using the coefficient of Gower. Cluster and principal coordinate analyses showed *Janthinobacterium lividum* to be a heterogeneous species but *Chromobacterium violaceum* and *C. fluviatile* to be well-separated and homogeneous phenons. The new, monospecific genus *Iodobacter* is proposed to accommodate *C. fluviatile*, which was originally placed in the genus *Chromobacterium*, despite its low guanine-plus-cytosine content (50 to 52 mol%, but 65 to 68 mol% for *C. violaceum*), pending the study of further isolates. The type strain of *Iodobacter fluviatile* comb. nov. is NCTC 11159.

Chromobacterium, a genus of violet-pigmented, gram-negative rods, was taxonomically unsatisfactory for many years, as it contained the two species *C. violaceum*, a fermentative mesophile, and *C. lividum*, a nonfermentative psychrophile. As Sneath (14) commented, "the species are almost as different from one another as are many genera". The ability of organisms to synthesize the same pigment, violacein, does not necessarily indicate a close relationship and should not be given more weight than any other taxonomic character. Indeed, the violacein-producing isolates described by Gauthier (5) had deoxyribonucleic acid (DNA) base compositions of only 41 mol% guanine plus cytosine, well outside the range of 63 to 72 mol% for the genus *Chromobacterium*. On the basis of ribosomal ribonucleic acid-DNA hybrid binding and stability studies, phenotypic data, and DNA-DNA hybridizations, De Ley et al. (4) concluded that the genus *Chromobacterium* should be split, and they placed the psychrophilic strains in the new genus *Janthinobacterium*.

Moss et al. (11) described violacein-producing isolates from river water which were fermentative and psychrophilic and which formed thin spreading colonies on low-nutrient media. These organisms shared several phenotypic properties with *C. violaceum* but formed a separate group in numerical taxonomic studies and had the much lower guanine-plus-cytosine content of 50 to 52 mol%, and the new species *C. fluviatile* was proposed. Subsequent ribosomal ribonucleic acid-DNA hybridization studies on the type strain of this species (9) indicated that it is taxonomically distinct from *C. violaceum* and *J. lividum*, but it was considered that *C. fluviatile* should be retained in the genus *Chromobacterium* until further strains had been isolated and studied, despite the discrepancy in DNA base ratios, and it appears so in the first edition of *Bergey's Manual of Systematic Bacteriology* (16).

The present work was undertaken to obtain further strains of *C. fluviatile*, which is infrequently isolated, to ascertain whether a new monospecific genus should be established to accommodate members of this species.

MATERIALS AND METHODS

Bacterial strains. Strains C001, C002, and C166 to C170 were *C. violaceum* NCTC 9757^T, F91 University of Surrey,

NCTC 9371, NCTC 9372, NCTC 9373, NCTC 9376, and NCTC 9695, respectively. Strains C003 and C004 were *Janthinobacterium lividum* NCTC 9796^T and F1308 University of Surrey, respectively. Strains C005 to C010 were *C. fluviatile* 162 (14), NCTC 11160, 163, 164, NCTC 11159^T, and NCTC 11161, respectively. Other reference strains were AM01 (*Aeromonas hydrophila* NCIB 9240), AM02 (*Aeromonas salmonicida* NCTC 10402), AS01 (*Alcaligenes faecalis* NCIB 8156), BA01 (*Bordetella bronchiseptica* NCTC 452), PL01 (*Plesiomonas shigelloides* NCIB 9242^T), PS01 (*Pseudomonas solanacearum* NCPPB 325), PS02 (*Pseudomonas acidovorans* NCIB 9681^T), PS03 (*Pseudomonas cepacia* NCIB 8507), PS04 (*Pseudomonas testosteroni* NCIB 8955^T), PS05 (*Pseudomonas aeruginosa* NCTC 10332^T), and VO01 (*Vibrio parahaemolyticus* NCMB 1164). *Janthinobacterium* strains C067, C070, and C071 were Antarctic soil core isolates B232.2 (Signy Island, South Orkney Islands) and MP5.5 and MP1.1 (Maiviken, South Georgia) (supplied by D. D. Wynn-Williams, British Antarctic Survey); C072 to C075 and C077 to C079 were soil isolates (C. Ryall strains, supplied by M. O. Moss, University of Surrey, Guildford, United Kingdom) 77A, 108, 130, 136, 145, 152, and 153; C057 and C060 were isolated from violet-pigmented composting eggshells. All other strains were isolated in Scotland from fresh water. C011 to C028, C030 to C044, C046, C048, C050, C053, C054, C058, C061 to C063, C081, C085 to C087, C093 to C095, C097, C098, C100, C102, C107 to C113, C117 to C120, C124, C126, C127, C130, C131, C134, C135, C137 to C139, C152 to C165, SUC1, and SUC3 were isolated from lowland burns in Baldernock, Stirlingshire, United Kingdom. C089 was isolated from Garb Uisge, Kilmahog, Stirlingshire. C142, C143, C147, C148, C150, and C151 were isolated from River Carron, Stirlingshire. C180 to C184 were isolated from Malls Myre, Glasgow (supplied by D. Cullen, Glasgow College of Technology). C185 to C194 were isolated from Clatteringshaws Loch (mean pH, 4.1), Kirkudbrightshire, United Kingdom. C195 was isolated from West Burn, Glasgow (supplied by D. Cullen).

Isolation and maintenance of strains. Strains of *Chromobacterium* and *Janthinobacterium* species were isolated by spreading 0.5- or 0.1-ml quantities of undiluted water samples on 14- or 9-cm plates containing the medium described by Ryall and Moss (12): Nutrient Broth no. 1 (Oxoid Ltd.

Basingstoke, Hampshire, United Kingdom) at one-quarter strength (1/4NB) solidified with 1.2% agar (Oxoid) (1/4 Nutrient Agar [1/4NA]) and supplemented with filter-sterilized solutions of colistin sulfate (Colomycin; Pharmax Ltd. Bexley, Kent, United Kingdom), cycloheximide, and sodium deoxycholate to give final concentrations of 15, 30, and 300 µg/ml, respectively. The medium described by Keeble and Cross (7) was also used initially but was found to be unsuitable for the isolation of *C. fluvatile*, which only grew from heavy inocula, forming plicated, nonspreading colonies. Plates were incubated at 25°C for 5 to 7 days, and violet colonies, especially those with spreading morphologies, were picked off, streaked on 1/4NA, and incubated at 25°C for 4 days to check purity. Strains SUC1, SUC3, C124, C126, C127, C130, C131, and C134 were unpigmented isolates recognized on the basis of colonial morphology and confirmed as *Chromobacterium* or *Janthinobacterium* species by flagellum staining and a limited number of biochemical tests. Pure cultures were transferred to 1/4NB, incubated at 25°C for 2 days, and then stored at 4°C. All strains were lyophilized in 5% meso-inositol nutrient broth.

Characterization tests and scoring of results. All media were inoculated with one drop of 1/4NB culture grown overnight at 25°C and, with the exception of those used for growth temperature tests, were incubated at 25°C. Media for strain VO01 were supplemented with 1% NaCl when necessary for growth. Results were scored in binary form (0, negative; 1, positive) or quantitatively (0, negative; 1, doubtful; 2, trace reaction; 3, weak positive; 4, moderate positive; 5, strong positive; or radii of inhibition zones in antibiotic sensitivity tests), the method used being indicated below after each test series.

Test control and reproducibility. Reference strains acted as positive and negative controls for most tests; other bacteria known to give either positive or negative reactions were included in the remaining tests to aid interpretation. Nine strains (C035, C058, C085, C102, C107, C118, C119, C185, and C193) were chosen at random and carried through in duplicate to ascertain test reproducibility.

Colonial morphology and pigmentation. Bacteria were grown on 1/4NA for 5 days and observed for concentric zoning of pigment (unpigmented strains were scored as no comparison); butyrous, gelatinous, tough, and membranous colonial textures; and spreading or plicated colonies (scored as 0 or 1). Bacteria were also grown on glucose yeast extract agar (containing [in grams per liter]: peptone, 2.5; NaCl, 2.5; yeast extract, 2.5; glucose, 5; agar, 12) adjusted to pH 7.0 and autoclaved at 115°C for 20 min for observation of violacein production (scored from 0 to 5), the identity of the pigment being confirmed by the nonspectrophotometric tests of Sneath (16), and for other pigments (scored as 0 or 1).

Microscopy. Overnight cultures in 1/4NB were examined by phase-contrast microscopy for straight rods, curved rods, rounded ends, pointed ends, and chains of more than two cells and were examined after 2 days for filament formation (scored as 0 or 1). Overnight cultures on slopes of 1/4NA were flagellum stained by using the method of Mayfield and Inniss (8) (with the proportion of alcoholic basic fuchsin in the mordanting solution raised from 0.8 to 0.95) and examined for lateral and polar flagella (scored as 0 or 1).

Growth tests. Abilities of strains to grow in 1/4NB at 4, 30, and 37°C were tested after 7, 4, and 2 days, respectively. Growth in 1/4NB with 1, 2, 4, and 6% NaCl, in 1/4NB at pH 3, 4, 5, and 6, on nutrient agar containing four times the usual concentration of Nutrient Broth (Oxoid) (4NA), on minimal medium (2) (scored as 0 or 1), and anaerobically on Nutrient Agar (Oxoid) in a GasPak jar (BBL Microbiology Systems, Baltimore, Md.) (scored as 0 to 5) were tested after 7 days.

Biochemical tests. The oxidase test was performed by the method of Sivendra et al. (13) (scored as 0 or 1). Oxidation (scored as 0 to 5) and fermentation (scored as 0 to 5) of

glucose and gas production from glucose (scored as 0 or 1) were tested in the glucose-H₂S medium of Ward et al. (18), which was examined daily for 5 days. Acid production from *N*-acetylglucosamine, L-arabinose, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, glycerol, glycogen, meso-inositol, inulin, lactose, D-maltose, D-mannitol, D-mannose, melezitose, D-raffinose, D-sorbitol, starch, sucrose, trehalose, or D-xylose was indicated by a yellow reaction in a deep-butted slope of medium containing the following substances (in grams per liter): (NH₄)₂PO₄, 1; KCl, 0.2; MgSO₄ · 7H₂O, 0.2; yeast extract, 0.2; agar, 12; and phenol red, 0.06. The medium was adjusted to a pH of 7.4 and autoclaved at 121°C for 15 min, and filter-sterilized carbohydrate solution was added to give a final concentration of 0.5%. Tubes were examined at 2, 4, and 7 days (scored as 0 to 5). Utilization of acetate, citrate, fumarate, glycerate, lactate, malate, propionate, pyruvate, succinate, or tartrate as the sole carbon source was tested in the medium of Gordon and Mihm (6) modified by increasing the concentration of phenol red to 0.06 g/liter; slopes were examined for red reactions at 2, 4, and 7 days (scored as 0 to 5). Hydrolysis of aesculin, arginine, and casein, the egg yolk reaction, and production of HCN were tested by the methods of Sneath (16) (scored as 0 to 5). Hemolysis on 5% horse blood in Columbia Blood Agar base (Oxoid) was examined after 2 days (scored as 0 to 5). Hydrolysis of gelatin and starch in nutrient agar plates were tested by the methods of Cowan and Steel (1) at 7 days (scored as 0 to 5). Nitrate reduction, destruction of nitrite, and gas production from nitrite were tested in nitrate broth (1) at 7 days using Merckoquant nitrate test strips (Merck, Darmstadt, Federal Republic of Germany).

Antibiotic sensitivities. Multidisks U2 and 30-12L (Oxoid) containing ampicillin (25 µg), cephaloridine (25 µg), colistin sulfate (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), nitrofurantoin (200 µg), sulfafurazole (500 µg), tetracycline (50 µg), chloramphenicol (10 µg), chlortetracycline (10 µg), furazolidone (50 µg), neomycin (10 µg), oxytetracycline (10 µg), streptomycin (10 µg), sulfafurazole (100 µg), and penicillin G (1.5 IU) were placed on 1/4NA lawn plates, and radii of zones of complete inhibition were measured (in millimeters) from the edges of the disks after 2 days (scored as 0 to 18). Sensitivity to filter paper disks containing 50 µg of vibriostatic agent 0/129 (2,4-diamino-6,7-diisopropylpteridine; Sigma Ltd., Poole, Dorset, United Kingdom) was determined on 1/4NA streak plates after 2 days (scored as 0 or 1).

Analysis of results. Similarities were calculated by using the general similarity coefficient, (*S_G*) of Gower, clusters formed by the unweighted pair group method using arithmetic averages, and principal coordinates computed by using the GENSTAT IV package on the Prime 9955 computer at the Glasgow College of Technology. Ordination plots were rotated by using the MACSPIN program (D² Software Inc., Austin, Texas) on an Apple Macintosh microcomputer.

RESULTS

The nine duplicates and their homologous strains showed similarities ranging from 98.9 to 89.5% *S*, with a mean of 95.5% *S*. This implies an overall test error of 4.5% (*P* ~2.25%), which is acceptably low (17), especially since many of the data were scored quantitatively.

Rotated principal coordinates plots of vectors 1, 2, and 3, which together accounted for 63% of the variation in the data (vector 1, 36%; vector 2, 17%; vector 3, 10%), for 70

FIG. 1. Rotated principal coordinate plots of the first three vectors of an analysis of 70 representative strains. ■, *C. fluviatilis*; □, *C. violaceum*; ▲, ▼, and △, *Janthinobacterium lividum*; ○, reference strains. Groups A through D are indicated. Panel b is a rotated view of panel a so that it is viewed from above.

representative strains are shown in Fig. 1. The computer program had a capacity of 70 strains, but different sets of strains gave similar results. Four main groups (A, B, C, and D) which remained stable when rotated may be recognized. In Fig. 1a, the reference strains lie in a diffuse band between group D and the other groups, and five strains of chromobacteria (C027, C028, C067, C163, and C195) lie at some distance from any other group. In Fig. 1b, the plot shown in Fig. 1a has been rotated so that it is viewed obliquely from above.

The phenogram (Fig. 2) shows that four clusters formed at the 56% *S* level, leaving one strain unattached. Cluster 1 contained all but three of the strains, including unpigmented ones, tentatively identified as *J. lividum* on isolation, and the type strain of the species. Seven smaller clusters (1.1 to 1.7) forming at between 70 and 80% *S* have been indicated to facilitate discussion; clusters 1.1 to 1.5 and the ungrouped

strains C085 to C147 correspond to group A in the principal coordinate plots.

Cluster 1.1 contained the type strain of *J. lividum* the one other culture collection strain of this species, and six strains (C011, C012, C050, C058, C117, and C139) isolated from water; members of the group shared no distinctive property. The 15 strains (C135, C137, C138, C143, C148, C150, C154, C155, C157-C159, C161, C164, C165, and C186) in cluster 1.2 were isolated from water, one of them from the acidified Clatteringshaws Loch, and the majority formed butyrous colonies with zoning of pigment. Cluster 1.3 contained the 19 isolates C044, C073, C077, C079, C097, C142, C151 to C153, C156, C160, C162, C180 to C184, SUC1, and SUC3 isolated from soil and water, all of which produced membranous colonies most of which became plicated with age; SUC1 and SUC3 were unpigmented isolates suspected to be *J. lividum* on the basis of their plicated, membranous colonies. Cluster 1.4 contained the 18 strains C013, C043, C048, C057, C060, C074, C075, C078, C086, C087, C095, C107, C108, C124, C126, C127, C131, and C134 isolated from soil, water, and egg shell membranes, and although members shared no distinctive character (as with cluster 1.1), one-third were unpigmented isolates. Cluster 1.5 comprised the three soil isolates C070 to C072, two of which came from South Georgia, and all produced gelatinous colonies. Strains C085 and C147 were isolated from water and produced tough, gelatinous colonies.

Clusters 1.6 and 1.7 correspond to group B in the ordination plots. The five members (C089, C093, C094, C098, and C100) of cluster 1.6 were water isolates which mainly produced gelatinous colonies which were tough and plicated in several strains. Cluster 1.7 contained isolates C187 to C194 from the acidified Clatteringshaws Loch; all produced tough, plicated, gelatinous colonies and shared with members of cluster 1.6 the distinctive properties of producing acid from glycogen and trehalose and failing to produce acid from polyalcohols (glycerol, inositol, mannitol, and sorbitol) or to hydrolyze aesculin. Results for several other tests such as hemolysis and growth in 1% NaCl and on minimal medium were usually negative for group B strains but were positive for group A. Members of these two clusters were not separated in the ordination plots. Strains C067, C163, and C195 were recognizable as *J. lividum*, but all grew poorly and produced weak acid reactions from few carbohydrates.

Cluster 2.1 contained 45 typical strains (C005 to C010, C014 to C026, C030 to C042, C046, C053, C054, C061 to C063, C081, C102, C111 to C113, C120, and C130) of *C. fluviatilis*, including the type strain, all of which produced spreading, butyrous colonies; all but two strains clustered above 80% *S*. The four strains C109, C110, C118, and C199 in cluster 2.2 formed only slightly spreading colonies (scored as nonspreading), and for two of them (C109 and C110) these were slightly gelatinous; a notable character of members of this cluster was a positive egg yolk reaction. Clusters 2.1 and 2.2 are not separated in the ordination plots, where they form group C. The two ungrouped strains (C027 and C028) formed discrete nonspreading colonies and were tentatively identified as *J. lividum* on isolation; C027 was unpigmented, and C028 fermented fewer carbohydrates, utilized fewer organic acids, and showed greater sensitivity to several antibiotics than did other strains of *C. fluviatilis*, and it lies at some considerable distance from other members of this taxon in the ordination plots. In the cluster analysis, members of *C. fluviatilis* merged by 68% *S*, with all but the two strains C027 and C028 merging by 79% *S*; this phenon joined

FIG. 2. Phenogram based on unweighted pair group method using arithmetic averages analysis of 140 chromobacteria and 11 reference strains. Clusters 1.1 to 1.5 and strains C085 and C147, *J. lividum*; 1.6, 1.7, and strains C195, C163, and C067, atypical strains of *J. lividum*; 2.1, 2.2, and strains C027 and C028, *C. fluviale*; 3, *C. violaceum*; AM01, *Aeromonas hydrophila*; VO01, *Vibrio parahaemolyticus*; PL01, *Plesiomonas shigelloides*; AM02, *Aeromonas salmonicida*; PS02, *Pseudomonas acidovorans*; PS04, *Pseudomonas testosteroni*; AS01, *Alcaligenes faecalis*; PS03, *Pseudomonas cepacia*; PS05, *Pseudomonas aeruginosa*; PS01, *Pseudomonas solanacearum*; and BA01, *Bordetella bronchiseptica*.

TABLE 1. Patterns of results in diagnostically useful tests

Property	% Positive in group ^a (no. of strains):				Results for ungrouped strain:				
	A (68)	B (14)	C (53)	D (9)	C063	C167	C195	C027	C028
Spreading colonies	0	0	89	0	-	-	-	-	-
Gelatinous colonies	38	100	4	0	+	+	+	-	-
Tough colonies	7	71	0	0	-	+	+	-	-
Growth at 4°C	97	93	100	0	-	+	+	+	+
Growth at 37°C	0	0	0	100	-	-	-	-	-
Growth on minimal medium	100	7	96	100	-	-	-	-	-
Anaerobic growth	3	0	100	100	-	-	-	-	-
Glucose fermented	0	0	100	100	-	-	-	+	+
Glucose oxidized	97	57	0	0	-	-	+	-	-
Acid from:									
L-Arabinose	100	100	0	0	-	-	-	-	-
D-Cellobiose	97	100	0	0	+	-	+	-	-
D-Galactose	100	93	0	0	+	+	-	-	-
Gluconate	1	0	96	100	-	-	-	+	+
Glycerol	98	0	13	100	-	-	+	-	-
Glycogen	3	100	0	89	-	-	-	-	-
meso-Inositol	100	0	0	0	-	-	-	-	-
D-Maltose	98	93	100	0	+	+	+	+	+
D-Mannitol	97	0	0	0	-	-	-	-	-
N-Acetylglucosamine	1	0	100	100	-	-	-	+	+
Trehalose	1	100	100	100	+	-	+	+	+
Lactate utilization	100	93	0	100	-	+	-	-	-
Aesculin hydrolysis	100	0	0	0	-	-	-	-	-
Arginine hydrolysis	0	0	0	100	-	-	-	-	-
HCN production	0	0	0	100	-	-	-	-	-

^a Groups: A, *J. lividum*; B, atypical *J. lividum*; C, *I. fluviatile*; D, *C. violaceum*. Groups were indicated by principal coordinate analysis.

the cluster containing *C. violaceum* at 55%, and both groups were well separated in the ordination plots, with strains of *C. violaceum* forming group D.

Cluster 3 contained eight strains of *C. violaceum*, C001, C002, C166 to C170, and C185, the last of these being a fresh isolate from Clatteringshaws Loch; all clustered above 85% *S* and formed a tight phenon. The reference strains of *A. hydrophila* and *V. parahaemolyticus* merged with *C. violaceum* at 67% *S*, with *Plesiomonas shigelloides* at 60.5% *S*, and with *A. salmonicida* at 58% *S*. Thus, the *C. violaceum* phenon showed greater similarity to these reference strains than it did to *C. fluviatile*.

Table 1 shows the patterns of results for the groups A, B, C, and D and the five ungrouped strains in the diagnostically useful tests; only results scored as 3 or higher in the quantitative tests were counted as positive.

The antibiotic sensitivity patterns and mean zone sizes were largely similar between the groups; the majority of strains were sensitive to chlortetracycline, kanamycin, nalidixic acid, and tetracycline, moderately sensitive to oxytetracycline, slightly sensitive to neomycin, streptomycin and sulfafurazole, and resistant to ampicillin, cephaloridine, colistin, penicillin G, and vibriostatic agent 0/129. The most noticeable differences were the resistance of *J. lividum* strains and the sensitivities of *C. violaceum* and *C. fluviatile* strains to nitrofurantoin and the greater sensitivities of *C. fluviatile* to chloramphenicol and of *C. violaceum* and *C. fluviatile* to furazolidone. The slight sensitivity of most strains to neomycin explains the unsuitability of the medium

of Keeble and Cross (7) for isolating certain types of *J. lividum* (19) and *C. fluviatile*.

DISCUSSION

Clusters 1.1 to 1.5, which merged between 70 and 80% *S*, may be regarded as a homogeneous group of typical *J. lividum* strains, with clusters 1.6 and 1.7 representing atypical strains corresponding to the former species "*C. amethystinum*" and "*C. membranaceum*". Moss and Ryall (10) found atypical strains of *J. lividum* to be more frequently isolated from soil, and so it is of particular interest that the proportions of typical and atypical strains are the same (84 and 16%, respectively) in the study of Moss et al. (11) and in the present work despite the different sources of the strains (discounting those from culture collections): 60% were from water, 24% were from riparian soils, and 16% were from river sediments in the earlier study, and 86% were from water and 14% were from soil in the present one.

The genus *Janthinobacterium* falls into two main groups in the principal coordinates analysis, but the picture is less distinct in the phenogram in which cluster 1.6 merges with clusters 1.1 to 1.5, which contain typical strains, at a higher *S* than that of its merger with cluster 1.7. Sneath (15) found that gelatinous (atypical) strains and other strains of *J. lividum* cross-reacted serologically and did not differ notably in other ways. The ribosomal ribonucleic acid-DNA hybridization studies of De Ley et al. (4) indicated one slightly elongated group rather than two groups, but it is unclear whether their collection of 11 cultures included strains which were truly atypical in the present sense. *J. lividum* must therefore continue to be regarded as a heterogeneous species.

The homogeneity of *C. violaceum* and the stabilities of its members are emphasized by the high similarities of the fresh isolate C185 to the "museum piece" strains from culture collections. Being a mesophilic saprophyte, *C. violaceum* is particularly associated with tropical environments, as are its opportunistic infections, called chromobacteriosis, of man and other mammals (review manuscript in preparation), but strains have been found in cooler habitats. Strain C185 came from acidified loch water which has a mean temperature of perhaps 9°C (range, 3 to 18°C), and isolates from Antarctic lakes and soils, some of which were acidic, have been reported (19). It is noteworthy, therefore, that all of the *C. violaceum* strains in this study grew in 1NB at pH 4, whereas the majority of strains of the other two species, including most of those of *J. lividum* from Clatteringshaws Loch (mean pH, 4.1), did not. Sneath reported no growth of *Chromobacterium* or *Janthinobacterium* species below pH 5 (15, 16), but Wynn-Williams (19) isolated acid-tolerant strains of *J. lividum* in the Antarctic and considered the pH range for polar habitats of this species to be from 3.5 to 9.0.

Most of the strains of *C. fluviatile* form a homogeneous group in cluster analysis and in principal coordinates analysis, and the two outlying or atypical strains, C027 and C028, are recognizably members of this species, which is well separated from *Janthinobacterium* species, *C. violaceum*, and the reference strains. Indeed, *C. violaceum* strains show greater similarity to reference strains of the family *Vibrionaceae* and members of rRNA superfamily I of De Ley (3) than they do to *C. fluviatile* or to members, such as *Alcaligenes faecalis*, *B. bronchiseptica*, *Janthinobacterium* species, *P. acidovorans*, *P. cepacia*, *P. solanacearum*, and *P. testosteroni*, of rRNA superfamily III, to which they belong.

C. fluviatile was placed in the genus *Chromobacterium* (11) as a matter of convenience pending the study of further

strains, as the guanine-plus-cytosine content of the species was too low for formal inclusion. The DNA-ribosomal ribonucleic acid hybridization studies of Moss and Bryant (9) further supported the recognition of a new genus for this species. The present work confirms both the homogeneity of the *C. fluviale* taxon and the need for it to be recognized as a genus separate from *Chromobacterium*; accordingly, the new genus *Iodobacter* is proposed to accommodate this species, and a description follows.

***Iodobacter* gen. nov.** *Iodobacter* gen. nov. (from Gr. adj. *ioeides* violet-colored; M. L. masc. n. *bacter*, the equivalent of Gr. neut. n. *bacterion*, a small rod; M.L. masc. n. *Iodobacter*, a violet-colored, small rod): gram-negative, straight, round-ended rods 0.7 by 3.0 to 3.5 μm , occurring singly, in pairs, sometimes in chains, and occasionally as long filaments. Motile with a single polar flagellum and one or more lateral flagella. Grow on ordinary peptone media. On low-nutrient media such as $\frac{1}{2}$ NA, colonies usually differ from those of *Chromobacterium* and *Janthinobacterium* species by being very thin, with rough surfaces and irregular edges, and spreading to 1 cm or more in diameter; they are of butyrous consistency and are easily emulsified in water. Occasional strains may spread only poorly and may produce slightly gelatinous colonies. All strains produce nonspreading colonies on rich media. Most strains produce the violet pigment violacein, and this shows most intensely in the centers of spreading colonies. Growth in $\frac{1}{2}$ NB is moderate after 24 h at 25°C, with uniform turbidity and a violet ring at the surface but no pellicle; strains forming long filaments may appear to gel in broths. Chemoorganotrophs; facultative anaerobes which attack carbohydrates fermentatively without gas production. Grow at 4 to about 30°C, with optimum growth around 25°C. *Iodobacter* species differ from *Chromobacterium* species by inability to grow at 37°C and from *Janthinobacterium* species by anaerobic growth and fermentative attack of carbohydrates. Other characters useful for separating these genera are shown in Table 1.

***Iodobacter fluviale* comb. nov.** *Iodobacter fluviale* comb. nov. has all the characters defining the genus. In addition, it has the following properties: positive for growth in 1% NaCl; positive for oxidase; production of acid from fructose, glucose, maltose, mannose, trehalose, and *N*-acetylglucosamine; utilization of malate as the sole carbon source; and hydrolysis of gelatin. Negative for growth in 4% NaCl and at pH 3; production of acid from arabinose, cellobiose, galactose, glycogen, inositol, inulin, lactose, mannitol, melezitose, raffinose, sorbitol, starch, sucrose, and xylose; utilization of lactate, propionate, or tartrate as the sole carbon source; hydrolysis of aesculin, arginine, and starch; production of hydrogen cyanide; reduction of nitrite; production of gas from nitrate. Sensitive to the following antibiotics: chloramphenicol, chlortetracycline, furazolidone, kanamycin, nalidixic acid, nitrofurantoin, oxytetracycline and tetracycline; slightly sensitive to neomycin, streptomycin, and sulfafurazole; resistant to ampicillin, cephaloridine, colistin, penicillin G, and vibriostatic agent 0/129. Variable properties (with proportion of strains giving positive results shown in parentheses) are: spreading colonies (89%), gelatinous colonies (4%), and zoning of pigment (6%) on $\frac{1}{2}$ NA; violacein production (83%); formation of chains of cells (34%) and filaments (15%); growth at 30°C (83%), in 2% NaCl (9%), and at pH 4 (19%) and pH 9 (98%); growth on minimal medium (96%) and 4NA (45%); production of acid from gluconate (96%) and glycerol (13%); utilization of acetate (89%), citrate (85%), fumarate (89%), glycerate (60%), pyruvate (79%), and succinate (94%); hydrolysis of casein (90%); hemolysis (92%); egg yolk reaction (13%); nitrate reduction (98%). The guanine-plus-cytosine contents of the DNA from three

strains ranged from 50 to 52 mol% as determined by melting temperature (11).

Isolated from running fresh water in England and Scotland, and reported (19) from Antarctic lakes. Strains of this species are infrequently isolated, and the selective medium of Ryall and Moss (12) is recommended, with subsequent cultivation on $\frac{1}{2}$ NA or in $\frac{1}{2}$ NB.

Description of the type strain. The type strain is NCTC 11159 (strain C009 in the present study) and has the characters of the species. With respect to variable characters, tests are positive for production of spreading colonies and violacein; growth at 30°C, on minimal medium, and at pH 5; production of acid from gluconate; utilization of acetate, citrate, fumarate, glycerate, pyruvate, or succinate as the sole carbon source; hydrolysis of casein; hemolysis; and nitrate reduction. Tests were negative for production of gelatinous colonies; zoning of pigment; formation of chains of cells; filamentous forms; growth in 2% NaCl, at pH 4, and on 4NA; production of acid from glycerol; and egg yolk reaction.

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