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Original Article

Isolation and characterization of marine luminous bacteria from shallow coastal waters of Taiwan

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Background and Purpose: Marine luminous bacteria were isolated and identified from samples in shallow coastal waters of Taiwan during the relatively warm seasons.
Methods: Identification of the luminous isolates was performed based on differences of phenotypic and genotypic characteristics together with data from phylogenetic analysis.
Results: Twenty seven strains of marine luminous bacteria were isolated. They were divided into five types based on differences of phenotypic characteristics. However, they could be clustered into only two genotypes according to the analysis of restriction patterns of polymerase chain reaction-amplified 16S rRNA genes digested with various restriction enzymes. The characterization data together with the 16S rRNA gene-based phylogenetic analysis revealed that the isolates included in phenotype I (seven isolates) could be *Photobacterium leiognathi*, and those included in phenotypes II-V (twenty isolates) might be classified as *Vibrio harveyi*. However, phylogeny based on *gyrB* sequences indicated that phenotypes II-V could be classified into two species, *V. harveyi* and *Vibrio campbellii*.
Conclusion: Culturable luminous bacteria in the shallow coastal waters of Taiwan during the sampling period are dominated by *V. harveyi*/*campbellii* and *P. leiognathi*, and the former species appeared to be more prevalent and numerous than the latter species in general.

Key words: Base sequence; Chemiluminescent measurements; *Photobacterium*; Seawater; *Vibrionaceae*

Introduction

Marine luminous bacteria (LB) are of great interest because of their unique trait of emitting visible light. They are ubiquitous in the marine environment, occupying a variety of planktonic (free-living), parasitic, saprophytic, gut-symbiotic (commensally enteric) and light organ-symbiotic (mutualistic) niches [1-4]. Eleven species in the genera *Vibrio*, *Photobacterium* and *Shewanella* are reported to contain marine LB. They can be divided into four groups according to DNA/DNA relatedness and phylogeny based on 16S rRNA gene sequences: (i) *Shewanella* group (*Shewanella hanedai*, *Shewanella woodyi*); (ii) *Photobacterium* group (*Photobacterium leiognathi*, *Photobacterium phosphoreum*); (iii) *Vibrio fischeri* group (*V. fischeri*, *Vibrio logei*); and (iv) *Vibrio harveyi* group (*V. harveyi*, *Vibrio vulnificus*, *Vibrio splendidus*, *Vibrio orientalis*, *Vibrio cholerae*) [5,6]. The LB included in the *Shewanella* group are capable of respiratory but not fermentative metabolism, whereas those included in the other groups can carry out both respiratory and fermentative metabolism.

V. harveyi (synonym, *Beneckea harveyi* or *Lucibacterium harveyi*) [7-9], *V. fischeri (*synonym, *Photobacterium fischeri*) [8], *P. leiognathi*, and *P. phosphoreum* constitute the majority of the luminous bacterial isolates that had been identified. These species may occur in seawater and the enteric tracts of marine animals. *V. fischeri*, *P. leiognathi* and *P. phosphoreum* are also found as specific symbionts in the light organs of various fishes and squids. In contrast, some *V. harveyi* strains are recognized pathogens for penaeid larvae and snooks in hatcheries [10].

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In the present study, 27 strains of marine LB were identified in water samples collected from the shallow coastal regions of northern and southern Taiwan. The isolation and characterization of these luminous bacterial strains are described.

Methods

Culture media, collection of water samples and isolation of LB

Culture media described by Shieh and Jean [11] were used for the present study. Modifications and additional media are described below. Peptone-yeast (PY) broth was modified to contain Bacto peptone at 3 g/L and Bacto yeast extract at 1 g/L, and pH was adjusted to 8.0. PY-II broth containing 3 g/L Bacto peptone and 1 g/L Bacto yeast extract was dissolved in 80% seawater, approximately 3.5% salinity, and was adjusted to pH 8.0. Bacto agar (Difco, Detroit, MI, USA) was added to PY broth and PY-II broth at 4 g/L and 15 g/L for the preparation of stab and plate media, respectively. PY-nitrate broth was prepared by adding potassium nitrate to PY broth at 2 g/L. Modified PY plate medium used for the lipase test was supplemented with calcium chloride (0.2 g/L) and Tween 80 (0.1%). PY-carbohydrate stab media differed from the original media in containing Bacto peptone, Bacto yeast extract, Bacto agar and bromothymol blue at concentrations of 3 g/L, 1 g/L, 8 g/L and 0.06 g/L, respectively. Modified Møller decarboxylase media contained 5 g/L of appropriate L-amino acid (L-arginine, L-lysine or L-ornithine). Tryptone broth was adjusted to pH 8.0. Glucose-mineral medium differed from that described by Shieh et al [12] in containing Tris at 3 g/L.

Nine water samples were collected at a depth of about 30 cm from seven stations set up in the shallow coastal regions of northern and southern Taiwan during the relatively warm seasons: 6 samplings from stations A, B, C and D in 2002, and 3 samplings from stations E, F and G in 2004 (Fig. 1). The water samples had original temperatures of 23-30°C, while the seawater temperature around the year in the sampling regions might vary from 16-30°C.

Each water sample was serially diluted with sterile seawater. Plate counts of LB and heterotrophic bacteria were done by spreading 0.1 mL aliquots of each dilution onto PY-II plates in duplicate or triplicate. Incubation was carried out at 25°C in the dark for 7 days under aerobic conditions. Luminous colonies produced on the plates were examined and marked daily in a dark room during the incubation. All of the luminous colonies were picked off and purified by successive streaking on PY-II plates. The isolates that again retained luminescence were maintained in PY-II stab medium and stored at 25°C.

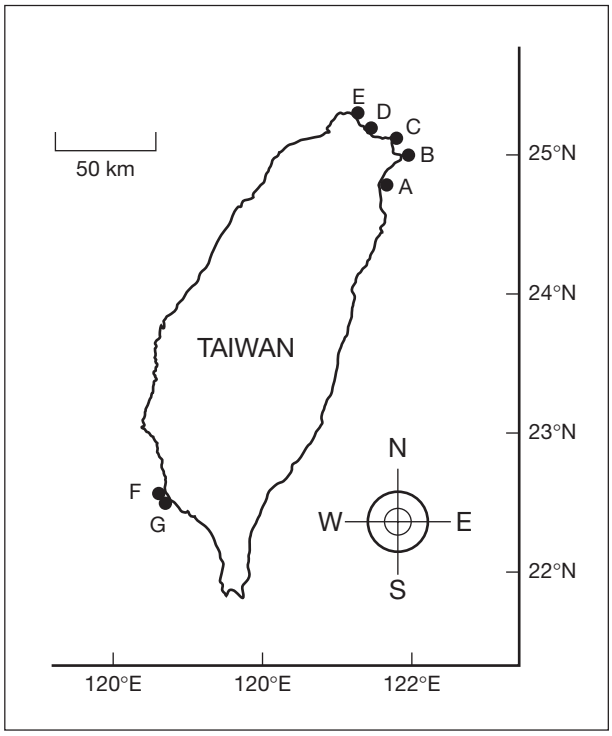


Fig. 1. Locations of water sampling stations (A, B, C, D, E, F, G).

Phenotypic characterization of luminous isolates

Cells grown in PY plate medium were sampled with a straight needle for inoculation into each of the PY-carbohydrate stab media for tests of acid production from fermentation of various carbohydrates. The surface of the medium in each tube was overlaid with sterile liquid paraffin after inoculation. The cultures were examined for color changes daily for 7 days. Gas, if produced would be indicated by formation of gas bubbles or cracks in the medium, or by the separation of medium from the side or bottom of the tube. The cultures for tests of arginine dihydrolase and lysine and ornithine decarboxylases were also incubated under anaerobic conditions by overlaying the surface of the medium in each tube with sterile liquid paraffin after inoculation [13].

Cells grown for 1-3 days on PY plate medium were used to test Gram reaction by the potassium hydroxide lysis method [14]. They were also used to test luminescence, agarase, catalase, oxidase and swarming according to the procedures described by Shieh et al [15]. Amylase, caseinase, DNase, gelatinase and lipase

tests essentially followed the methods of Smibert and Krieg [13]. Cells grown in PY broth for 1-3 days were examined for shape and motility by phase-contrast microscopy. Cultures grown in PY-nitrate broth were examined for nitrate reduction [16] and denitrification [17] daily for up to 5 days. Indole production was determined by Kovac's method [13] after incubating the tryptone broth cultures for 2-5 days. The ability to grow at different temperatures was determined in PY broth and recorded after incubating the cultures for 20 days at 4°C, and 3-7 days at 20-42°C. The ability to grow at various sodium chloride (NaCl) levels was determined in PY broth containing 0-8% NaCl. Utilization of various compounds as sole carbon and energy sources for growth was determined in glucose-mineral medium and its modifications containing test substrates used in place of glucose; sugars and sugar alcohols were both provided at 5 g/L, while organic acids were provided at 2 g/L. All of the test cultures were aerobically incubated at 25°C in the dark for 7 days unless stated otherwise.

Restriction patterns of 16S rRNA genes and polymerase chain reaction amplification

PY broth cultures of the luminous isolates were aerobically incubated at 25°C in the dark for 24-36 h. Each culture was centrifuged to harvest the cells. Total genomic DNA was extracted and purified from the cells by using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA) in accordance with the instructions of the manufacturer. Hydration solutions of the purified DNA samples were prepared at concentrations in the range 200-700 μg/mL. The DNA hydration solutions were used for polymerase chain reaction (PCR) amplification.

PCR amplification of 16S rRNA genes was performed according to the procedures of Jean et al [18]. Aliquots (5 μL) of each of the PCR products of 16S rRNA genes were separately digested with restriction endonucleases *Sca*I (Takara, Japan), *Dde*I (Promega, Madison, WI, USA), *Hha*I (Takara) and *Rsa*I (Promega) according to the manufacturer's instructions. Restricted DNAs were analyzed by electrophoresis at 50 V for 60 min on 2% agarose gels in Tris-acetate-ethylenediamine tetra-acetic acid (TAE) buffer. After electrophoresis, the gels were stained with ethidium bromide (1 μg/mL) for 10 min in TAE buffer. DNA bands appearing on the gels were visualized and photographed under an image analyzing system consisting of an ultraviolet trans-illuminator (Spectroline, Westbury, NY, USA), a dark box (EDAS 290, Kodak, New Haven, CT, USA) and a room digital camera (DC290, Kodak).

A PCR method based on the 38-bp repetitive extragenic palindromic (REP) sequence in *Enterobacteriaceae* and other bacteria [19] was also applied to the typing of the luminous isolates.

Sequencing and phylogenetic analysis

Sequencing reactions of 16S rRNA gene samples, alignment and comparison of the resulting sequences and reference sequences available in the GenBank database, calculation of the distance matrices for the aligned sequences and reconstruction of the phylogenetic dendrogram were as described by Shieh et al [20]. Sequences of *gyrB* were determined using the methods described by Ast and Dunlap [21]. Phylogenetic analysis of *gyrB* sequences essentially was the same as the analysis of the phylogeny for the 16S rRNA genes used in the present study.

DNA-DNA hybridization

DNA-DNA hybridization experiments were performed among *V. harveyi* American Type Culture Collection (ATCC) 14126^T, *Vibrio campbellii* ATCC 25920^T and some of the luminous isolates. Bacterial DNA was isolated and extracted using the Puregene DNA isolation kit. The DNA was blotted on a Hybond-N$^+$ membrane (Amersham Pharmacia Biotech, Arlington Heights, IL, USA) by use of a Bio-Dot microfiltration apparatus (Bio-Rad Laboratories, Hercules, CA, USA), thereafter dot-blot hybridization was done at 68°C [22]. Total DNA of either ATCC 14126^T or ATCC 25920^T labeled with a DIG DNA labeling kit (Roche Diagnostics, Basel, Switzerland) was used as a probe. Hybrid detection was performed by enzyme immunoassay and enzyme-catalyzed color reaction using a DIG nucleic acid detection kit (Boehringer Mannheim, Mannheim, Germany). DNA relatedness values among ATCC 14126^T, ATCC 25920^T and the luminous isolates were estimated according to the hybridization results examined using the 1D Image Analysis Software (Kodak).

Results and Discussion

Enumeration and isolation of LB

LB were found in all of the nine water samples taken from stations A-G. Plate counting values of these bacteria ranged from 7 to 500 colony-forming units (CFU) per mL, which accounted for 0.5-6.9% of the total CFU of heterotrophic bacteria (Table 1). Previous reports have shown that LB were of densities ranging from lower than 10^0 to 10^1 CFU per mL in nearshore

Table 1. Plate counts of luminous bacteria and heterotrophic bacteria in shallow coastal waters of Taiwan

Sampling information					Heterotrophic bacteria (total CFU/mL)	Luminous bacteria (luminous CFU/mL)
No.	Station	Date	Temperature (°C)	Salinity (%)		
1	A	Apr 2002	28	3.15	1.0×10^4	2.5×10^2
2	B	Jul 2002	25	3.20	1.1×10^3	1.3×10^1
3	C	Jun 2002	30	3.10	7.3×10^3	3.3×10^1
4	C	Sep 2002	29	3.45	1.4×10^3	1.3×10^1
5	D	Sep 2002	28	3.70	5.0×10^3	3.3×10^1
6	D	Oct 2002	23	3.85	1.3×10^3	7.0×10^0
7	E	Jul 2004	30	3.40	5.1×10^4	2.7×10^2
8	F	Aug 2004	30	1.35	5.8×10^3	4.0×10^2
9	G	Aug 2004	30	2.34	7.7×10^3	5.0×10^2

Abbreviation: CFU = colony-forming units

waters [8,9,23-26]. The luminous CFU obtained from the water samples in the present study were generally greater than those obtained in the previous reports. This might be attributed to the different water temperature conditions. Eight out of the nine water samples counted here had original temperatures of 25-30°C, while in those previous reports, the original temperatures were rarely higher than 25°C. Differences in the water temperature have been correlated to the density of certain species of LB in seawater [24,26-28].

Twenty seven strains of LB were isolated from the counting plates. They were designated as LB1 to LB27, respectively. After subculturing, strains LB1-LB16 remained luminous, while the luminescence of strains LB17-LB27 was no longer visible. Losses of phenotypic features after subculture have also been observed in other bacteria, such as the loss of catalase activity [29], denitrifying activity [17,30], and the ability to produce gas vesicles [31], fascicle-like colonies [32] and square tablets of 8 to 64 cells [33]. Gene deletion was assumed to account for the occurrence of some of these phenomena [17,30]. Whether gene deletion could explain the loss of luminescence in our isolates remains to be further investigated.

Phenotypic characterization of luminous isolates

All of the luminous isolates were motile, Gram-negative rods that produced flat and off-white colonies on PY plates. They were halophiles, unable to grow in the absence of NaCl. They were facultative anaerobes, capable of both respiratory and fermentative metabolism; acid but no gas was produced during fermentation of glucose. Oxidase and catalase tests were both positive. All of the isolates might be identified as members of the family *Vibrionaceae* according to the preliminary phenotypic characterization [34,35]. However, they were classified into five types based on more detailed phenotypic characteristics (Table 2).

Phenotype I contained strains (LB1-LB3 and LB17-LB20) that did not ferment cellobiose, trehalose and mannitol, and were unable to grow on these substrates as sole carbon and energy sources. The strains of this phenotype were negative in the tests for indole production and amylase, DNase, gelatinase, and lipase activities.

Phenotypes II-V contained isolates that fermented cellobiose and trehalose and grew on these substrates as sole carbon and energy sources. Phenotypes II and III contained strains that grew at 40°C. The strain of phenotype II (LB4) differed from those of phenotype III (LB5-LB12 and LB21-LB22) in that it produced arginine dihydrolase and was unable to grow on mannitol as a sole carbon and energy source. Phenotypes IV (LB13-LB14 and LB23-LB26) and V (LB15-LB16 and LB27) contained strains that did not grow at 40°C. However, only the strains of phenotype IV grew in 8% NaCl and did not grow on inositol and malonate as sole carbon and energy sources.

Restriction pattern analysis of 16S rRNA genes and REP-PCR typing

The 16S rRNA gene sequences of all luminous isolates were PCR-amplified with the primer pair mentioned above. All of the isolates produced a single band of about 1500 bp, corresponding to the predicted size of the 16S rRNA gene sequences amplified from this primer pair.

Restriction patterns of the PCR-amplified 16S rRNA genes were analyzed by digestion with the restriction endonucleases *Dde*I, *Hha*I, *Rsa*I and *Sca*I. Only one restriction pattern, designated pattern a, was obtained with *Dde*I, while two patterns, designated patterns a and b, respectively, were obtained with each of the other

Table 2. Phenotypic characteristics of luminous bacterial isolates in comparison with those of the type strains of *Vibrio harveyi, Vibrio campbellii* and *Photobacterium leiognathi*[a]

Characteristic	Bacterial phenotype and strain number included					*P. leiognathi* ATCC 25521^T	*V. harveyi* ATCC 14126^T	*V. campbellii* ATCC 25920^T
	I (7)	II (1)	III (10)	IV (6)	V (3)			
Amylase	–	+	+	+	+	–	+	+
Caseinase	–	+	V	+	+	–	ND	ND
DNase	–	+	+	+	+	ND	+	ND
Gelatinase	–	+	+	+	+	–	+	+
Lipase	–	+	+	+	+	+	+	+
Arginine dihydrolase	–	+	–	–	–	+	–	–
Lysine decarboxylase	V	+	+	+	+	–	+	+
Ornithine decarboxylase	–	+	+	+	+	–	+	–
Indole production	–	+	+	+	+	–	+	+
Growth at (temperature)								
4°C	V	–	–	–	–	–	–	–
20-35°C	+	+	+	+	+	+	+	+
40°C	–	+	+	–	–	–	+	–
42°C	–	–	–	–	–	–	–	–
Growth in (salinity)								
0% NaCl	–	–	–	–	–	–	–	–
1-6% NaCl	+	+	+	+	+	+	+	+
8% NaCl	–	+	V	+	–	–	–	–
Fermentation source								
Glucose	+	+	+	+	+	+	+	+
D-Arabinose	–	–	–	–	–	–	–	–
L-Arabinose	–	–	–	–	–	–	–	–
Cellobiose	–	+	+	+	+	–	–	+
Mannose	V	+	+	+	+	+	+	+
Trehalose	–	+	+	+	+	–	+	+
Dulcitol	–	–	–	–	–	–	–	+
Mannitol	–	–	+	+	+	–	+	+
Sole carbon source								
Glucose	+	+	+	+	+	+	+	+
D-Arabinose	–	–	–	–	–	–	–	–
L-Arabinose	–	–	–	–	–	–	–	–
Cellobiose	–	+	+	+	+	–	+	+
Mannose	+	+	+	+	–	+	+	+
Trehalose	–	+	+	+	+	–	+	+
Xylose	–	–	–	–	–	–	–	–
Dulcitol	–	–	–	–	–	–	–	–
Inositol	–	–	–	–	+	–	–	–
Mannitol	–	–	+	+	+	–	+	+
Acetate	V	+	+	+	+	+	–	–
Citrate	–	+	+	+	+	–	+	+
Fumarate	+	+	+	+	+	ND	ND	ND
Malonate	–	–	–	–	+	–	–	–
Tartrate	–	–	–	–	–	–	–	–

Abbreviations: ATCC = American Type Culture Collection; NaCl = sodium chloride; + = positive; – = negative; V = variable between strains; ND = no data available

[a]All of the luminous isolates and the type strains are motile, Gram-negative rods that are positive for oxidase, catalase and nitrate reduction and are negative for swarming, agarase, denitrification and gas from glucose fermentation.

Table 3. Genotypes of luminous isolates derived from restriction patterns of 16S rRNA genes digested with various restriction enzymes

Genotype	Phenotype	*Dde*I	*Hha*I	*Rsa*I	*Sca*I
I	I	a	a	a	a
II	II-V	a	b	b	b

restriction enzymes (Table 3). Two different genotypes of 16S rRNA genes were detected in the luminous isolates by analyzing the restriction patterns. Genotype I contained the seven isolates of phenotype I. Genotype II consisted of the twenty isolates of phenotypes II-V (Table 3). However, eleven REP-PCR typing patterns (3-15 amplified bands ranging in size from 250-3500 bp) were discernible in the luminous isolates LB1-LB16 (Fig. 2). This indicated that REP-PCR typing might be more effective than 16S rRNA genotyping in differentiation of the luminous isolates.

Phylogenetic analysis

For the phylogenetic analysis of 16S rRNA gene and *gyrB* sequences, strains LB1, LB4, LB5, LB13, and LB15 were selected as representative strains of the phenotypes I-V, respectively. Almost complete 16S rRNA gene sequences of these strains (1433-1443 bp) were determined. The *gyrB* sequences of LB1, LB4, LB5 and LB13 were obtained at 1169-1180 bp, while that of LB15 was not determined successfully. Gene sequences that had been determined were aligned and compared with all bacterial sequences available in the GenBank database. The GenBank accession numbers for the 16S rRNA gene sequences of LB1, LB4, LB5, LB13, and LB15 were DQ14934-DQ14938 and those for the *gyrB* sequences of LB1, LB4, LB5 and LB13 were DQ499006-DQ499009. Our phylogenetic analysis confirmed that all of the representative strains were members of the family *Vibrionaceae* in the gamma-*Proteobacteria*. Fig. 3 shows the 16S rRNA gene-based phylogenetic positions of these strains within the radiation of their related taxa in the family *Vibrionaceae*. Strain LB1 clustered with species of the genus *Photobacterium*. Its closest relatives were *P. leiognathi* subsp. *mandapamensis* (99.3% sequence similarity) and *P. leiognathi* subsp. *leiognathi* (98.6% sequence similarity) [21]. No other *Photobacterium* species shared more than 97% sequence similarity with the strain. Strains LB4, LB5, LB13 and LB15 had sequence similarity levels of 97.6-99.4%. They formed a cluster at sequence similarity levels of 97.6-99.8% with *V. harveyi* and other *Vibrio* species, including *V. campbellii*, *Vibrio natriegens*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus* and *Vibrio proteolyticus*. Fig. 4 shows the *gyrB*-based phylogenetic relationships between strains LB1, LB4, LB5 and LB13 and other related members in the family *Vibrionaceae*. Again, strain LB1 clustered with species of the genus *Photobacterium*, with *P. leiognathi* subsp. *mandapamensis* (98.7% sequence similarity) and *P. leiognathi* subsp. *leiognathi* (97.6% sequence similarity) as its closest relatives. However, LB4, LB5 and LB13 fell into two separate clusters. LB4 and LB5 clustered with *V. campbellii* at sequence similarities of 97.1-98.2%, and LB13 clustered with *V. harveyi* at a sequence similarity of 98.6%. The sequence similarities between strains of the two clusters were only 93.0-94.4%.

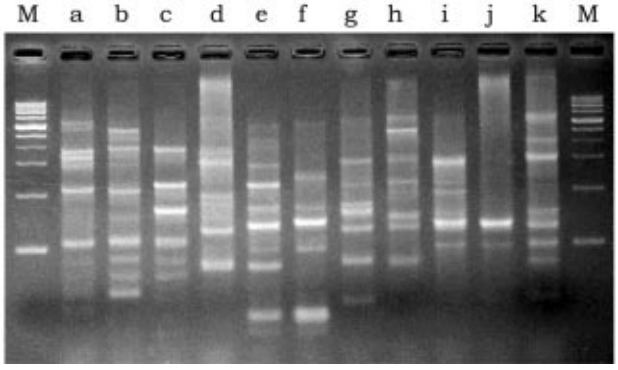


Fig. 2. Diagram of amplification patterns of selective luminous isolates with repetitive extragenic palindromic sequence-polymerase chain reaction method. Patterns: a, LB1; b, LB2; c, LB3; d, LB4, LB7, LB9, LB10 and LB11; e, LB5; f, LB6; g, LB8; h, LB12; i, LB13 and LB16; j, LB14; k, LB15. M, molecular size markers (from top to bottom: 10,000, 8000, 6000, 4000, 3000, 2500, 2000, 1500, 1000, and 500 bp).

A prokaryote whose 16S rRNA gene sequence differs by more than 3% from that of all other organisms is generally considered as a new species, and sequence differences of greater than 5-7% (93-95% identity) have been taken as support for a new genus [36]. Strain LB1 could be identified as *P. leiognathi*, since the two had rather similar sequences (similarity > 98.5%) and shared high similarity (93.3-97.8%) in their phenotypic characteristics (Table 2). Such identification was also supported by high levels of *gyrB* sequence similarity between LB1 and *P. leiognathi*. The high 16S rRNA sequence similarity levels of strains LB4, LB5, LB13 and LB15 with *V. harveyi*, *V. campbellii*, *V. natriegens*, *V. alginolyticus*, *V. parahaemolyticus* and *V. proteolyticus* indicated the phylogenetic impracticability for differentiation of *V. harveyi*,

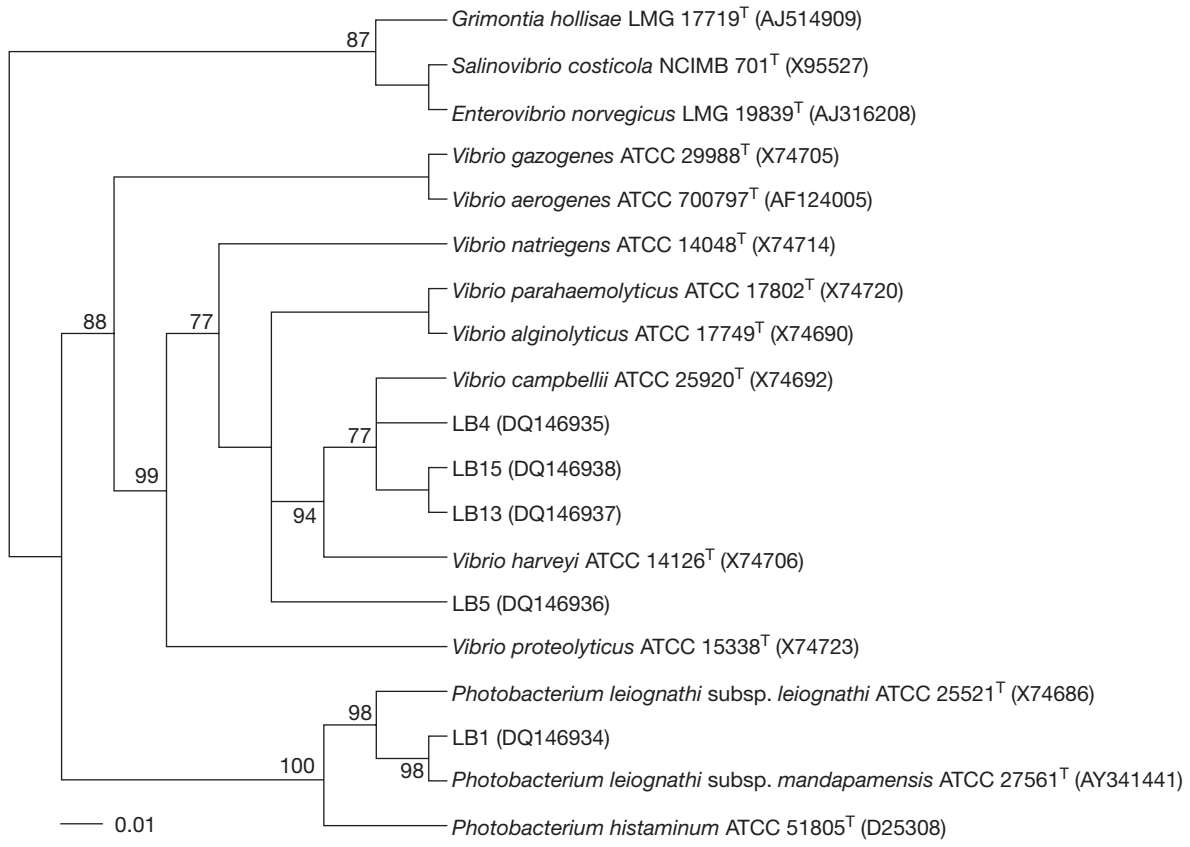
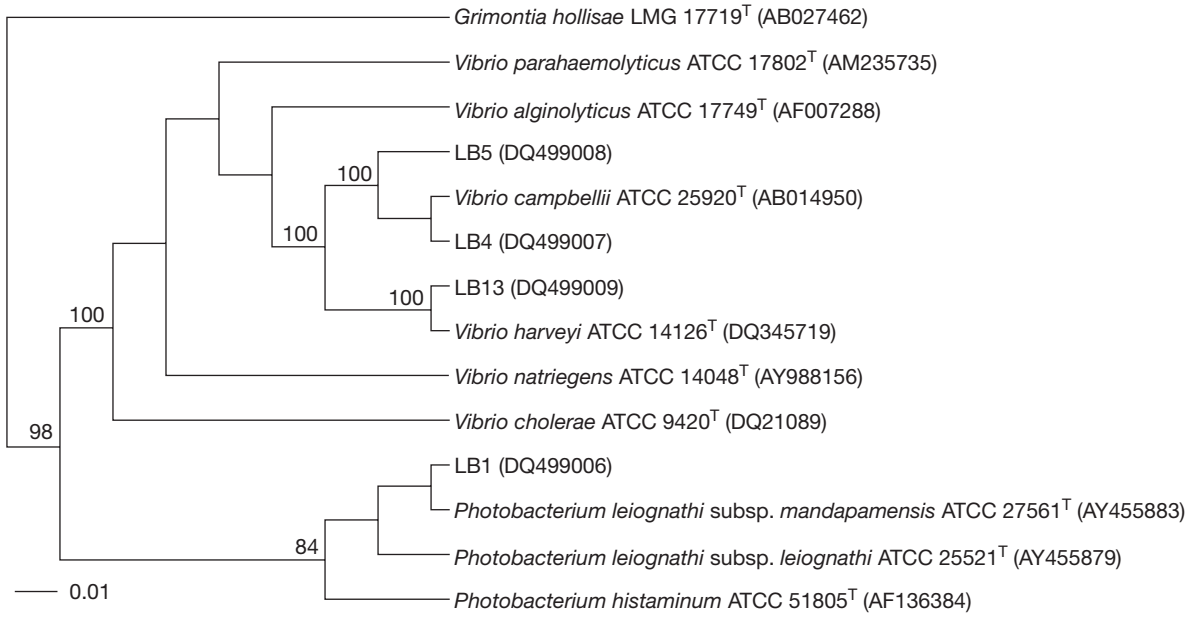


Fig. 3. Unrooted phylogenetic tree deduced from neighbor-joining analysis of the 16S rRNA gene sequences of selective luminous isolates and other related taxa in the family *Vibrionaceae*. Bootstrap confidence values (percentages) obtained with 100 re-samplings are given at the branch points; values below 75 are not shown. Bar = one nucleotide substitution per 100 nucleotides. LMG = Laboratorium voor Microbiologie, Universiteit Gent (UGent); NCIMB = National Collections of Industrial Food and Marine Bacteria; ATCC = American Type Culture Collection; LB = luminous bacteria.

V. campbellii, *V. natriegens*, *V. alginolyticus*, *V. parahaemolyticus* and *V. proteolyticus* and for identification of the four luminous isolates at the species level. However, the four luminous isolates might be classified as *V. harveyi* considering that only *V. harveyi* was defined to include LB in these *Vibrio* spp. [37]. Such classification was supported by high DNA-DNA relatedness values (68.2-88.8%) between the four strains and *V. harveyi* ATCC 14126^T. However, both LB4 and LB5 had higher DNA-DNA relatedness values with *V. campbellii* ATCC 14126^T than with *V. harveyi* ATCC 14126^T (LB4, 88.8% vs 80.7%; LB5, 81.0% vs 74.3%). This together with phylogeny based on *gyrB* sequences (Fig. 4) indicated that LB4 and LB5 are best classified as *V. campbellii* rather than *V. harveyi*. Our work provides the first evidence that *V. campbellii* may include LB. It also suggests that the *gyrB*-based phylogeny could easily differentiate *V. harveyi* from *V. campbellii*, despite the phenotypical and genomical similarity of the two species [37,38].

The Microtox® test, based on the inhibition of light emission of *V. fischeri*, is a practical method for monitoring the toxicity of aquatic samples. This test is considered one of the most sensitive, rapid and reliable microbial bioassays applicable for detection of water quality [39]. Microbiosensor B17 677F, a biotest based on the luminescence quenching of *P. phosphoreum*, is also useful for assessing the toxicity of sewage and river water [40]. The application of the present luminous isolates in tests of water quality and toxicity remains to be evaluated.

Previous studies based on phenotypic characterization have shown that *V. harveyi* and *V. fischeri* are generally the most frequently encountered species of LB in temperate nearshore seawaters. The two species accounted for more than 90% of the LB isolated from Sargasso Sea waters at depths of 160-320 m, where *P. leiognathi* and *P. phosphoreum* constituted the remainder of the isolates [7]. They even occupied 99% of the luminous isolates from California coastal surface



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waters, where *V. harveyi* predominated (60-70%) in the summer, but was almost completely replaced by *V. fischeri* during the winter [8]. Fifteen strains of LB isolated from Tyrrhenian Sea coastal waters off northeastern Sicily were characterized by a combination of phenotypic and molecular tests. All of the strains were identified to be members of *V. harveyi* [5].

The present work showed that culturable LB in the shallow coastal waters of Taiwan during the relatively warm sampling seasons would be dominated by *V. harveyi*/*campbellii* and *P. leiognathi*, and that the former species (*V. harveyi*/*campbellii*) appeared to be more prevalent and numerous than the latter species in general (Table 4). Culturable LB distributed in temperate and tropical nearshore waters may comprise few predominant species other than *V. harveyi*/*campbellii*, *V. fischeri* and *P. leiognathi*. The species distribution patterns of these bacteria, however, may vary greatly due to geographical and seasonal differences.

Table 4. Distribution of luminous strains of various species in the sampling stations

Sampling		No. of luminous strains[a]	
No.	Station	*Vibrio harveyi/ campbellii*	*Photobacterium leiognathi*
1	A	5 (3)	0
2	B	1 (1)	1 (1)
3	C	1 (1)	0
4	C	1 (0)	0
5	D	0	3 (2)
6	D	2 (0)	0
7	E	3 (0)	1 (1)
8	F	5 (1)	0
9	G	2 (1)	2 (0)

[a]Numbers in parentheses show the strains that lost visible luminescence after subculture.

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