NOTE

***Photobacterium histaminum* Okuzumi *et al.* 1994 is a later subjective synonym of *Photobacterium damsela* subsp. *damsela* (Love *et al.* 1981) Smith *et al.* 1991**

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The type strain of *Photobacterium histaminum*, JCM 8968^T (= ATCC 51805^T), and that of *Photobacterium damsela* subsp. *damsela*, ATCC 33539^T, exhibit 100% identity in their 16S rRNA sequence, more than 80% DNA–DNA homology and only one phenotypic difference. Also, like *P. histaminum*, *P. damsela* subsp. *damsela* was shown to excrete a large amount of histamine when cells were grown on medium containing excessive histidine under acidic conditions. Therefore, the name *P. histaminum* should be considered to be a later subjective synonym of *P. damsela* subsp. *damsela*.

Keywords: *Photobacterium histaminum*, *Photobacterium damsela* subsp. *damsela*, 16S rDNA sequence, histamine production

Photobacterium damsela was initially isolated as *Vibrio damsela* from naturally occurring skin ulcers of temperate-water damselfish (Love *et al.*, 1981) and was recognized as an opportunistic pathogen capable of causing disease in a variety of hosts, including sharks (Grimes *et al.*, 1984), dolphins (Fujioka *et al.*, 1988) and humans (Morris *et al.*, 1982). MacDonell & Colwell (1985) transferred this species to the new genus *Listonella* based on a review of phylogenetic relationships within the family by 5S rRNA sequence data. Smith *et al.* (1991) further transferred this species to the genus *Photobacterium* based on phenotypic data. Also, Gauthier *et al.* (1995) have shown that the notorious fish pathogen *Pasteurella piscicida* NCIMB 2058^T was extremely closely related to *P. damsela* ATCC 33539^T on the basis of a phylogenetic analysis of small-subunit rRNA sequences and DNA–DNA hybridization data. Accordingly, *P. damsela* was proposed to include two subspecies, *Photobacterium damsela* subsp. *damsela* and *Photobacterium damsela* subsp. *piscicida*, though a study (Thyssen *et al.*, 1998) on the detailed morphological and biochemical characteristics of these two subspecies has shown that they are clearly distinguishable and there is no phenotypic evidence to include *P. damsela* subsp. *piscicida* as a subspecies of *P. damsela*.

In 1994, a new species named *Photobacterium histaminum* was described by Okuzumi *et al.* (1994) as a halophilic potent histamine-producing bacterium. The species was isolated during a survey of histamine-producing bacteria from marine fish and coastal waters as potential causative agents of scombroid fish poisoning (Okuzumi *et al.*, 1981; Yoguchi *et al.*, 1990a, b). The new species has been distinguished from other members of the genus (*Photobacterium phosphoreum*, *Photobacterium leiognathi*, *Photobacterium angustum*) based on phenotypic characteristics, including its ability to produce a large amount of histamine, 16S rRNA gene sequence comparison and DNA–DNA hybridization data. However, *P. damsela* subsp. *damsela* was not included as a reference organism in the latter study because a study of partial 16S rRNA sequences (Kita-Tsukamoto *et al.*, 1993) showed that *P. damsela* subsp. *damsela* together with *Vibrio costicola* and *Vibrio hollisae* belonged to a different lineage from the *Photobacterium* cluster. Also, a comparison with the partial sequence of the 16S rRNA gene of *P. damsela* subsp. *damsela*, available when the article by Okuzumi *et al.* (1994) was published, indicated 10 base differences in the 535 positions compared, corresponding to a level of similarity of 98.1%. Furthermore, phenotypic information available for *P. damsela* subsp. *damsela* (Smith *et al.*, 1991) indicated some phenotypic differences (e.g. amylase production, utilization of

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of strains JCM 8968^T and ATCC 33539^T are AB032014 and AB032015, respectively.

Table 1. Levels of DNA–DNA reassociation between *Photobacterium* strains

Source of unlabelled DNA	Relatedness (%) to labelled DNA from:	
	JCM 8968 ^T	ATCC 33539 ^T
<i>P. histaminum</i> JCM 8968 ^T (= ATCC 51805 ^T)	100	80
<i>P. damsela</i> subsp. <i>damsela</i> ATCC 33539 ^T	88	100
<i>P. damsela</i> subsp. <i>piscicida</i> NCIMB 2058 ^T	98	95
<i>P. angustum</i> NCIMB 1895 ^T	22	28
<i>P. leiognathi</i> NCIMB 2193 ^T	36	27
<i>P. phosphoreum</i> ATCC 11040 ^T	35	30

lactose and pyruvate) between this species and *P. histaminum*.

At present, the genus *Photobacterium* is proposed to include six species including two subspecies: *P. phosphoreum*, *P. leiognathi*, *P. angustum*, *P. histaminum*, *P. damsela* subsp. *damsela*, *P. damsela* subsp. *piscicida* and *Photobacterium iliopiscarium*. Phenotypically, these species can be differentiated from members of the genus *Vibrio* by the absence of a flagellar sheath (though *P. damsela* subsp. *piscicida* lacks flagella) and the accumulation of poly- β -hydroxybutyrate (PHB) during growth on glucose coupled with the inability to utilize the exogenous monomer of PHB as sole carbon source for growth, a property that is of importance in the definition of the genus *Photobacterium* (Baumann & Baumann, 1981). Recently, *Vibrio iliopiscarius* (Onarheim *et al.*, 1994) has been proposed to be reclassified as *P. iliopiscarium* comb. nov. on the basis of a phylogenetic study of almost the entire 16S rRNA gene sequence (Urakawa *et al.*, 1999), though the presence of a flagellar sheath and the accumulation of PHB for this species has not been established. The results of phylogenetic analysis performed with a nearly complete 16S rRNA sequence indicated that *Photobacterium* species together with *Vibrio costicola* and *Vibrio hollisae* can be differentiated from the genus *Vibrio* (Ruimy *et al.*, 1994; Gauthier *et al.*, 1995). Recently, *Vibrio costicola* has been proposed to be transferred to the new genus *Salinivibrio* because of a distinct phylogenetic position (16S rDNA sequence) from other *Vibrio* as well as *Photobacterium* species (Mellado *et al.*, 1996).

A close physiological similarity between *P. damsela* subsp. *damsela* and *P. histaminum* has been reported (Dalgaard *et al.*, 1997). Also, while we were working on a study of histamine-producing bacteria, a strain of halophilic, potent histamine-producing bacteria from marine fish was identified as *P. damsela* subsp. *damsela* from a partial sequence of the 16S rRNA gene. We obtained the *P. damsela* subsp. *damsela* reference strain (ATCC 51805^T) and analysed the histamine-producing ability of the species. We found that the type strain of this species has a histamine-producing ability as potent as *P. histaminum*. With the

existing 16S rRNA gene sequence data (Gauthier *et al.*, 1995; Okuzumi *et al.*, 1994), the overall level of sequence identity between *P. damsela* subsp. *damsela* and *P. histaminum* is 98.6% (20 base differences in 1436 positions), a value which must be considered in the context of possible uncertainties associated with 16S rRNA sequencing errors due to the differences in researchers and methods. To see whether these two species really are phylogenetically different, we decided to do comparative 16S rRNA and DNA–DNA hybridization analyses as well as a biochemical analysis. Since *P. histaminum* is relatively common in Japanese fish intestine and coastal waters (Yoguchi *et al.*, 1990a, b), it is also interesting from an ecological point of view if *P. histaminum* is closely related to the fish pathogen *P. damsela* subsp. *damsela*.

The type strains *P. phosphoreum* ATCC 11040^T and *P. damsela* subsp. *damsela* ATCC 33539^T were obtained from the American Type Culture Collection, Manassas, VA, USA. The type strains *P. damsela* subsp. *piscicida* NCIMB 2058^T, *P. angustum* NCIMB 1895^T and *P. leiognathi* NCIMB 2193^T were obtained from the National Collection of Industrial and Marine Bacteria, Aberdeen, UK. The type strain *P. histaminum* JCM 8968^T (= ATCC 51805^T) was isolated from marine fish and has been maintained in our laboratory. The strains were stabbed into 50% (v/v) artificial seawater (ASW)/peptone/yeast extract agar containing 0.5% polypeptone, 0.3% yeast extract and 1.5% (w/v) agar in 50% (v/v) ASW (pH 7.0) (Okuzumi *et al.*, 1981), and were maintained at 15 °C. The inocula used for phenotypic tests were prepared from 18- to 24-h-old cultures in 50% (v/v) ASW/PYG broth containing 1% (w/v) Bacto-peptone, 0.3% yeast extract and 0.3% glucose in 50% (v/v) ASW (pH 7.0), incubated at 30 °C.

In this study, the *P. damsela* subsp. *damsela* ATCC 33539^T and *P. histaminum* JCM 8968^T 16S rDNA sequences were analysed. 16S rDNA amplification, purification and sequencing were performed using the MicroSeq 16S rRNA Gene Kit (Applied Biosystems) as recommended by the manufacturer with a model ABI 310 automatic DNA sequencer (Applied Biosystems). The near complete 16S rDNA sequences

were determined from overlapping sequence data by using the GENETYX computer program (Software Development). The 16S rDNA sequences between positions 43 and 1429 (*Escherichia coli* numbering) of *P. damsela*e subsp. *damsela*e ATCC 33539^T and *P. histaminum* JCM 8968^T were identical in all positions.

As the two species could not be differentiated based on their 16S rRNA gene sequences, the genetic relatedness between the two species was studied by DNA–DNA hybridization experiments using other members of the genus: *P. phosphoreum*, *P. leiognathi*, *P. angustum* and *P. damsela*e subsp. *piscicida*. Cells were suspended in Tris/EDTA buffer (pH 8.0) and treated with lysozyme (final concentration, 2 mg ml⁻¹) and SDS (final concentration, 0.5%) for lysis. Chromosomal DNA was purified by standard methods (Sambrook *et al.*, 1989), except that the final steps of the procedure were modified as described by Satomi *et al.* (1997). DNA–DNA homology was studied by microplate hybridization methods (Ezaki *et al.*, 1989) with photobiotin labelling and colorimetric detection as described by Satomi *et al.* (1997). Table 1 shows the relatedness of the genomic DNAs of the strains tested. The levels of DNA relatedness between the type strains of *P. histaminum* and *P. damsela*e subsp. *damsela*e ranged from 80 to 88% and a high relatedness of 98% was also found between *P. histaminum* and *P. damsela*e subsp. *piscicida*. These values are higher than the DNA similarity threshold value recommended for species delineation (70%) (Wayne *et al.*, 1987). Both species showed less than 40% relatedness with other members of *Photobacterium*, the results being consistent with those of a previous study (Okuzumi *et al.*, 1994).

From a survey of the literature, it is apparent that phenotypic differentiation of these organisms is difficult. Okuzumi *et al.* (1994) reported that *P. histaminum* differed from *P. damsela*e subsp. *damsela*e (Smith *et al.*, 1991) in amylase production and utilization of lactate and pyruvate. Dalgaard *et al.* (1997) added the utilization of acetate and maltose for distinguishing these two species. However, conflicting data on these phenotypic traits have been reported: (i) amylase production by *P. damsela*e subsp. *damsela*e was negative in the study by Smith *et al.* (1991), but positive according to Gauthier *et al.* (1995) and Thyssen *et al.* (1998); and (ii) utilization of acetate and maltose was negative for *P. damsela*e subsp. *damsela*e in the study by Dalgaard *et al.* (1997), but positive according to Gauthier *et al.* (1995). Such discrepancies cast doubt as to whether these traits can differentiate these organisms properly at the phenotypic level. In this study, comparison of physiological characteristics was done by using API 20E, API CH50 and API ZYM (bioMérieux). Additional biochemical tests were performed as described by Okuzumi *et al.* (1994). The results indicated that both species were positive for amylase production and the utilization of lactate and pyruvate (with 0.05% yeast extract supplied in the basal medium), acetate and maltose. Other physiological properties tested by API 20E, API ZYM and

API CH50 were confirmed to be identical for both species, except trehalose utilization (negative for *P. damsela*e subsp. *damsela*e), the only physiological difference found between the two species. Apparently, this difference is not a criterion for the differentiation of two species because it is likely to result from mutational events. In addition, *P. damsela*e subsp. *damsela*e was found to produce a large amount of histamine when cells were grown in medium containing histidine under acidic conditions (pH 5.5) as *P. histaminum* does.

As described above, we could find no significant difference to justify separation of the type strain of *P. histaminum* and that of *P. damsela*e subsp. *damsela*e phenotypically and genotypically. Therefore, we propose that *P. histaminum* Okuzumi *et al.* 1994 be a later subjective synonym of *P. damsela*e subsp. *damsela*e (Love *et al.* 1981) Smith *et al.* 1991.

Emended description of *Photobacterium damsela*e subsp. *damsela*e

The original description of the species *Photobacterium damsela*e subsp. *damsela*e given by Smith *et al.* (1991) is unchanged, except that this subspecies is positive for the production of extracellular amylase. In addition, this subspecies is able to excrete a large amount of histamine into extracellular medium containing histidine under acidic conditions (pH 5.5).

Acknowledgements

We thank the laboratory of Applied Biosystems, Japan, for providing ABI 310 automatic DNA sequencers and technical assistance for analysis of 16S DNA sequences. The study was supported in part by a Grant-in-Aid for Scientific Research (A08556034) from the Ministry of Education, Science, Sports and Culture of Japan and a Grant-in-Aid from the Japan Food Industry Center.

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