Revisiting the genus Photobacterium: taxonomy, ecology and pathogenesis

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Summary. The genus Photobacterium, one of the eight genera included in the family Vibrionaceae, contains 27 species with valid names and it has received attention because of the bioluminescence and pathogenesis mechanisms that some of its species exhibit. However, the taxonomy and phylogeny of this genus are not completely elucidated; for example, P. logei and P. fischeri are now considered members of the genus Aliivibrio, and previously were included in the genus Vibrio. In addition, P. damselae subsp. piscicida was formed as a new combination for former Vibrio damsela and Pasteurella piscicida. Moreover, P. damselae subsp. damselae is an earlier heterotypic synonym of P. histaminum. To avoid these inconveniences draft and complete genomic sequences of members of Photobacterium are increasingly becoming available and their use is now routine for many research laboratories to address diverse goals: species delineation with overall genomic indexes, phylogenetic analyses, comparative genomics, and phenotypic inference. The habitats and isolation source of the Photobacterium species include seawater, sea sediments, saline lake waters, and a variety of marine organisms with which the photobacteria establish different relationships, from symbiosis to pathogenic interactions. Several species of this genus contain bioluminescent strains in symbiosis with marine fish and cephalopods; in addition, other species enhance its growth at pressures above 1 atmosphere, by means of several high-pressure adaptation mechanisms and for this, they may be considered as piezophilic (former barophilic) bacteria. Until now, only P. jeani, P. rosenbergii, P. sanctipauli, and the two subspecies of P. damselae have been reported as responsible agents of several pathologies on animal hosts, such as corals, sponges, fish and homeothermic animals. In this review we have revised and updated the taxonomy, ecology and pathogenicity of several members of this genus. [Int Microbiol 20(1): 1-10 (2017)]

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Taxonomic and phylogenetic perspectives

The genus Photobacterium has a long standing in microbiology, having received attention for more than one century by the bioluminescence that some of its species exhibit. Indeed, etymologically it means light producing bacterium. To date, it contains 27 species with valid names (Table 1). The historical development of the taxonomy of this genus is relatively easy to follow. The type species, Photobacterium phosphoreum, was included in the Approved Lists of Bacterial Names [79] together with P. angustum, P. (Aliivibrio) fischeri and P. leiognathi. The only species described in the
following decade, *P. logei*, is now considered a member of the genus *Aliivibrio* and so is *P. fischeri* [64]. In turn, *P. damsela* [80] was formed as a new combination for former *Vibrio damsela* and *Pasteurella piscicida*. This species is the only one for which subspecies have been proposed so far with the publication of *P. damsela* subsp. *piscicida* [27]. Moreover, *P. damsela* subsp. *damsela* is an earlier heterotypic synonym of *P. histaminum* [37,60]. In the last two decades the pace of descriptions has intensified with the proposal of 20 novel species and two new combinations with valid names (Table 1), which gives an average of about two new species names per year. According to minute 17 of the Subcommittee meetings on the taxonomy of *Aeromonadaeae*, *Vibrionaceae* and related organisms held in Istanbul, Turkey, in 2008 [31], the type strain of *P. aphylicae* is not available and a neotype strain has not been proposed to date.

At the time of validation [79], the description of the genus was the one given in the 8th edition of Bergey's Manual. Although an emendation has never been formally proposed it has been revised and updated recently [84].

**Phylogeny.** *Photobacterium* is one of the nine genera contained in the family *Vibrionaceae* (order “Vibrionales”, class *Gammaproteobacteria*). It is also the largest one after the type genus *Vibrio*. Following a practice that is common and more developed for *Vibrio* spp. [64,73], several authors have established different clades within the genus *Photobacterium* [6,73,86]. Clades are usually named after the older species name, referring to its validation date, regardless of the position of that strain into the clade. Currently, four clades have been described in the genus *Photobacterium: Damsela* (*P. damsela* subsp. *damsela* and *P. damsela* subsp. *piscicida*), *Phosphoreum* (*P. angustum*, *P. aquimarins*, *P. iliopiscarium*, *P. kishitanii*, *P. leognathi*, *P. phosphoreum* and *P. piscicola*), *Profundum* (*P. aestuarii*, *P. aphylicae*, *P. frigidiphilum*, *P. indicum*, *P. lipoleticum*, *P. profundum*, *P. sanguinicancrini* and *P. swingsii*), and *Rosenbergii* (*P. aphoticum*, *P. ganghwense*, *P. halotolerans*, *P. jeanni*, *P. lutimaris*, *P. marinum*, *P. rosenbergii*). But the clustering of *P. aquae*, *P. gaetbulicola*, *P. galatheae*, *P. palaluri*, and *P. sanctipauli* has not been elucidated yet. It has to be noted that this classification into clades has no standard in nomenclature although it can make more amenable the study of large genera by grouping together lines of descents. However, this achievement requires the application of robust molecular approaches and large sets of strains (not just type strains). A comprehensive study meeting both requirements is still pending to the best of our knowledge but at least it is optimistic to see that most recent species descriptions include phylogenetic analysis using alternative genes [48,56,71,83] or MLSA schemes [7,10,26,29,45,50,92]. This means that at least for some genes there are sequences available in public repositories for most (ideally all) the type strains and even for a number of additional isolates of some of them.

The genes more frequently employed to perform phylogenetic studies within the genus *Photobacterium* are *recA* (protein RecA, recombinase A), *rpoA* (RNA polymerase α subunit), *gyrB* (DNA gyrase subunit B), *pyrH* (uridine monophosphate kinase), *gapA* (glyceraldehyde-3-phosphate dehydrogenase, NAD-dependent glyceraldehyde-3-phosphate dehydrogenase), *ftsZ* (cell division protein FtsZ), *topA* (DNA topoisomerase I), and *mreB* (rod shape-determining protein MreB).

At the same time, draft and complete genomic sequences of members of the genus *Photobacterium* are increasingly becoming available and their use is becoming routine for many research laboratories to address diverse goals: species delineation with overall genomic indexes, phylogenetic analyses, comparative genomics, and phenotypic inference [2,29,50]. At the time of writing the present review (1 March, 2017), a search at the Assemblies database in NCBI [http://www.ncbi.nlm.nih.gov/] shows that there are 67 results for *Photobacterium*, 15 of which are from strains flagged as type material. A more careful examination reveals that two of these can be considered redundant entries (they are from two equivalent designations of the same strain, the type strain of *P. damsela*, sequenced in different laboratories) and another one is from “*P. marinum*” that has not been validated to date. Since there are 27 species in the genus, the resulting 13 genomic sequences represent about half of them. Thus the gap to be filled to give full coverage to the type strains of the genus in terms of availability of their genomic sequences is not too large and we can anticipate it might be reached soon. Although most of these genomic sequences are assembled into contigs or scaffolds, there are two completed, *P. gaetbulicola* Gung47T and *P. profundum* SS9.

One advantage of having large data sets of genomes is that they can be explored to search for the most suitable single gene phylogenetic marker. This objective has been addressed at the family level by Machado and Gram [49] who concluded that the *fur* (ferric uptake regulator Fur) gene was suitable for
that purpose and even developed a PCR method to be used for the amplification and sequencing of the gene. Phylogenetic analysis can also be a method to elucidate horizontal gene transfer as it was performed in the study by Urbanczyk et al. [85], who assessed the incidence of interspecies transfer of the lux genes (luxCDABEG), which encode proteins involved in luminescence and concluded that horizontal transfer of the lux genes in nature is rare and that horizontal acquisition of the lux genes apparently has not contributed to speciation in recipient taxa.

### Ecology of Photobacterium

The members of the genus *Photobacterium* thrive worldwide in oceans and show substantial ecophysiological diversity including free-living, symbiotic, piezophilic, and parasitic life styles. The habitats and isolation source of these species include seawater, sea sediments, saline lake waters, and a variety of marine organisms with which the photobacteria establish different relationships, from symbiotic ones, such as commensalism or mutualism, to pathogenic interactions.

### Table 1. Species, habitats and geographic sources of *Photobacterium* species

<table>
<thead>
<tr>
<th>Species</th>
<th>Habitats</th>
<th>Geographic sources</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aestuarii</em></td>
<td>Tidal flat sediment</td>
<td>Yeongam Bay (R. Korea)</td>
<td>[46]</td>
</tr>
<tr>
<td><em>P. angustum</em></td>
<td>Seawater</td>
<td>North Pacific Ocean (20°30'N 157°30'E)</td>
<td>[79]</td>
</tr>
<tr>
<td><em>P. aphoticum</em></td>
<td>Seawater</td>
<td>Malvarrosa beach, Valencia (Spain)</td>
<td>[48]</td>
</tr>
<tr>
<td><em>P. aplysiae</em></td>
<td>Eggs of sea hare (<em>Aplysia kurodai</em>)</td>
<td>Mogiyeo (R. Korea)</td>
<td>[75]</td>
</tr>
<tr>
<td><em>P. aquae</em></td>
<td>Malabar grouper (<em>Epinephelus malabaricus</em>)</td>
<td>Tianjin (China)</td>
<td>[45]</td>
</tr>
<tr>
<td><em>P. aquisinus</em></td>
<td>Seawater</td>
<td>Sagami Bay (Japan)</td>
<td>[92]</td>
</tr>
<tr>
<td><em>P. damselae</em></td>
<td>Damselfish (<em>Chromis punctipinnis</em>) skin ulcer</td>
<td>California (USA)</td>
<td>[47,80]</td>
</tr>
<tr>
<td><em>P. frigidophilum</em></td>
<td>Deep-sea sediments (1450 m)</td>
<td>Edison Seamount (western Pacific Ocean)</td>
<td>[74]</td>
</tr>
<tr>
<td><em>P. gaetbulicola</em></td>
<td>Tidal flat</td>
<td>Gungharbour (R. Korea)</td>
<td>[36]</td>
</tr>
<tr>
<td><em>P. galatheae</em></td>
<td>Mussel</td>
<td>Solomon Sea (Solomon Islands)</td>
<td>[50]</td>
</tr>
<tr>
<td><em>P. ganghwanense</em></td>
<td>Seawater</td>
<td>Ganhwa Island (R. Korea)</td>
<td>[63]</td>
</tr>
<tr>
<td><em>P. halotolerans</em></td>
<td>Water from a subterranean saline lake</td>
<td>Lake Martel, Mallorca (Spain)</td>
<td>[71]</td>
</tr>
<tr>
<td><em>P. iliotiscivorum</em></td>
<td>Intestines of fish (herring, coal fish, cod and salmon) living in cold seawater</td>
<td>Norway</td>
<td>[84]</td>
</tr>
<tr>
<td><em>P. indicum</em></td>
<td>Marine mud (400 m depth)</td>
<td>Indian Ocean</td>
<td>[32]</td>
</tr>
<tr>
<td><em>P. jeani</em></td>
<td>Healthy corals (<em>Palythoa caribaerum, Phylogorgia dilatata and Merulina ampliata</em>)</td>
<td>Brazil and Australia</td>
<td>[10]</td>
</tr>
<tr>
<td><em>P. kishitanii</em></td>
<td>Light organs and skin of several marine fish species</td>
<td>Japan, Cape Verde, Hawaii, Florida, South Africa</td>
<td>[7]</td>
</tr>
<tr>
<td><em>P. leiognathi</em></td>
<td>Light organ of teleostean fish (<em>Leiognathus</em>)</td>
<td>Gulf of Thailand (Thailand)</td>
<td>[66]</td>
</tr>
<tr>
<td><em>P. lipolyticum</em></td>
<td>Intertidal sediment</td>
<td>Yellow Sea (R. Korea)</td>
<td>[91]</td>
</tr>
<tr>
<td><em>P. lutimaris</em></td>
<td>Tidal flat sediment</td>
<td>Saemankum (R. Korea)</td>
<td>[33]</td>
</tr>
<tr>
<td><em>P. panulirii</em></td>
<td>Eggs of spiny lobster (<em>Panulirus penicillatus</em>)</td>
<td>Andaman Sea (India)</td>
<td>[13]</td>
</tr>
<tr>
<td><em>P. phosphoreum</em></td>
<td>Skin of marine animals, intestines of marine fish, luminous organs, seawater</td>
<td>Hawaii (USA), Japan and other locations</td>
<td>[79]</td>
</tr>
<tr>
<td><em>P. piscicola</em></td>
<td>Skin and intestine of marine fish, spoiled packed cod</td>
<td>North Sea (Holland), Denmark, Aberdeen Bay (UK)</td>
<td>[26]</td>
</tr>
<tr>
<td><em>P. profundum</em></td>
<td>Deep-sea sediment (5110 m)</td>
<td>RyukyuTrench (24°15.23’N 126°47.30’E)</td>
<td>[58]</td>
</tr>
<tr>
<td><em>P. rosenbergii</em></td>
<td>Tissue and water extracts of coral species</td>
<td>Magnetic Island (Australia)</td>
<td>[83]</td>
</tr>
<tr>
<td><em>P. sanctipauli</em></td>
<td>Coral (<em>Madracis decactis</em>)</td>
<td>St. Peter &amp; St. Paul Archipelago (Brazil)</td>
<td>[56]</td>
</tr>
<tr>
<td><em>P. sanguinicancri</em></td>
<td>Crab (<em>Maja brachydactyla</em>) haemolymph, mussels (<em>Mytilus edulis</em>)</td>
<td>Spain, Netherlands</td>
<td>[29]</td>
</tr>
<tr>
<td><em>P. swingsii</em></td>
<td>Pacific oysters (<em>Crassostrea gigas</em>), crab (<em>Maja brachydactyla</em>) haemolymph</td>
<td>Mexico, Spain</td>
<td>[28]</td>
</tr>
</tbody>
</table>

Additional strains are reported in Smith et al. [80] from human puncture wound, diseased shark, diseased turtle, diseased fish, aquarium seawater and fish surface.
Generally, in the marine environment (seawater and sediment), the species of *Photobacterium* are free-life forms, but they may colonize several animal surfaces developing neutral or negative relationships with the host. These nonspecific or pathogenic associations contrast with the highly specific, mutually beneficial association of certain *Photobacterium* species in bioluminescent symbiosis with aquatic animals [17].

There is not a clear discrimination between the *Photobacterium* species regarding to their relationship with the isolation source or habitat (Table 1). Thus, most of the nonluminous photobacteria (lack of lux operon genes) have been isolated from marine waters or sediments, but several strains of these species have been described in association with diseased or healthy corals, zoanthids, sea hares, mollusks, crabs and fish [28,29,39,45,69,75,83]. Nevertheless, strains of luminous *Photobacterium* species harbouring genes for luminescence (*lux CDABEG*) [19], such as *P. kishitanii*, *P. leiognathi*, *P. phosphoreum* and *P. piscicola*, have also been isolated from squids, corals and fish [6,26,34]. Therefore, the luminescence production property is not a key ability of this bacterial group to the specific colonization of none habitats, excepting the light-organs of squids and fish.

**Photobacteria as symbiotic of light-organs.** Several species of this genus contain bioluminescent strains including *P. angustum*, *P. aquimarís*, *P. damselae*, *P. ganghwaense*, *P. kishitanii*, *P. leiognathi*, *P. phosphoreum*, and *P. piscicola*. From them, *P. kishitanii* and *P. leiognathi* establish bioluminescent symbiosis with marine fish, squid and octopus [57]. These associations are typically highly specific at the animal family-bacterial species level; *P. leiognathi* with families *Leiognathidae*, *Acropomatidae* and *Apopogonidae* (Perciformes), and *Moridae* (Gadiformes) [21,34,82,88]; and *P. kishitanii* with the fish families *Chloropthalmidae* (Aciopiformes), *Macrouridae*, *Sleindachneridae* and *Moridae* (Gadiformes), *Trachichthyidae* (Beryciformes), *Opisthoprectidae* (Gemeroformes) and *Acropomatidae* (Perciformes) [6,20]. The animals accumulate dense populations of luminous bacteria in gland-like tissue complex called light organs [24], providing them with nutrients and oxygen for their growth and light production. The bacterial light in symbiotic animals is associated with sex-specific signalling, predator avoidance, locating or attracting prey, to name a few [82,86]. Symbiotic luminous bacteria have not an obligatory dependency of the host for their reproduction [23], but it seems that exist certain specificity between the symbiotic fish and the luminous *Photobacterium* species. The animals that establish a relationship with *P. leiognathi* as light-organ symbionts tend to be found in shallower waters, whereas the fish that are symbiotic with *P. kishitanii* are usually found in deeper waters [23,34]. This apparent specificity, which presumably would have a genetic basis, is believed to result from the host animal selecting its species of symbiotic bacteria and preventing that other bacteria could colonize its light organs. Several authors have proposed that the bioluminescent symbiosis might involve co-evolutionary interactions [21,86], due to the animal dependence of the bacterial light, its specialized anatomical adaptations for harbouring bacteria, and the host family-bacterial species specificity.

Although bioluminescent associations appear to be highly specific, in some cases two *Photobacterium* species may be present within individual light organs of fish [23,34], representing a phenomenon named cosymbiosis. Furthermore, different species of the same fish family sometimes harbour different *Photobacterium* species or even bacteria belonging to other bacterial genera, like *Aliivibrio* or *Vibrio* [23,24]. In addition, distinct strains of a single species may be present with individual light organs of both adult and larval fish [22,24]. This species- and strain-level variation demonstrates the lack of strict specificity in bioluminescent symbiosis.

Bioluminescent symbioses of fish and squid with luminous bacteria apparently do not exhibit codivergence (cospeciation), since phylogenies for host and their symbiotic bacteria present no meaningful topological congruence [23,34]. The patterns of symbiont-host affiliation in bioluminescent symbioses observed from nature therefore are not likely to have arisen through coevolutionary interactions. However, the absence of nonluminous bacteria in light-organs of fish and squid indicates that some kind of selection must be operative, like the environmental congruence [30]. The congruence between the environmental distribution of a predominant species of luminous bacteria and the fish developmental stage at which its light-organ is receptive to bacterial colonization, determines which bacterial species and strains establish the symbiosis [23,34]. Some environmental factors, such as the temperature, influence the abundance of the different species of luminous bacteria in the marine environment. Thus, lower temperatures found in deeper waters favour the prevalence of psychrotrophic species like *P. kishitanii*, which is the main light-organ symbiont in these waters. On the contrary, warmer waters favour the growth of mesophilic *Photobacterium* species, such as *P. leiognathi*.
being fish larvae in these waters more receptive to acquire these bacteria as light-organ symbionts.

In short, bioluminescent symbioses, therefore, differ from endosymbiotic associations, which are mutually obligate relationships in which the symbiotic bacteria are housed intracellularly and are transferred maternally. Symbiotic luminous bacteria are housed extracellularly, and in most cases they are known not to be obligately dependent on the host for their reproduction. Unlike obligate intracellular bacteria, the symbiotic luminous bacteria colonize a variety of other marine habitats, including intestinal tracts, skin, and body fluids of marine animals, sediments, and seawater, where they coexist and compete with many other kinds of microorganisms. A second major difference with endosymbiotic associations is that symbiotic luminous bacteria are acquired from the environment with each new generation of the host instead of being transferred vertically through the maternal inheritance mechanisms. Another major difference between bioluminescent symbiosis and endosymbiosis is that luminous bacteria and their host animals show no evidence of co-speciation. Endosymbiosis is generally assumed to involve coevolutionary interactions, that is, reciprocal genetic changes in host and symbiont that result from the obligate and mutual dependence of each partner on the other. Detailed molecular phylogenies of bacterially luminous fish and squids, however, are very different from the phylogenies of their symbiotic light-organ bacteria [18]. This lack of host-symbiont phylogenetic congruence demonstrates that the evolutionary divergence of symbiotic luminous bacteria has occurred independently of the evolutionary divergence of their host animals.

Bioluminescent symbioses appear to represent a paradigm of symbiosis that differs fundamentally from associations involving obligate, intracellularly transferred symbionts. While fish and squids are dependent ecologically on luminous bacteria, the bacteria are not obligately dependent on their bioluminescent hosts. The evolutionary adaptations for bioluminescent symbiosis, for example presence of light organs, accessory tissues for controlling, diffusing, and shaping the emission of light, and behaviour associated with light emission, all are borne by the animal. No genetic adaptations have been identified in the bacteria that are necessary for and specific to their existence in light organs compared to the other habitats they colonize. Therefore, luminous bacteria seem to be opportunistic colonizers, able to persist in animal light-organisms as well as in a variety of other habitats to which they are adapted.

Other question unanswered is regarding to the benefit of luminescence for the non-symbiotic photobacteria. This question has not been elucidated fully, but several explanations have been arisen. One of the most commonly cited explanations is that the bioluminescence increases the propagation and dispersal of bacteria by attracting fish or other marine animals to consume luminous material. This hypothesis based mostly on the prevalence of luminous bacteria in fish gut has not been demonstrated experimentally. Nevertheless, Zarubin et al. [93] established that zooplankton that contacts and feeds on P. leiognathi starts to glow, and the glowing individuals are highly vulnerable to predation by nocturnal fish. Glowing photobacteria are transferred to the intestines of fish and zooplankton, when they survive digestion and gain effective means for growth and dispersal. The use of bioluminescence, therefore, appears to be highly beneficial for marine bacteria, especially in oligotrophic areas of the deep sea.

Deep-sea sediments as habitats of Photobacterium species. Members of the genus Photobacterium are common inhabitants of marine waters sediments, including P. aequorum, “P. atraremae”, P. frigidiphilum, P. gaethuli-cola, P. indicum, P. lipolyticum, P. lutimaris, “P. marinum”, P. phosphoreum, and P. profundum. From them, P. frigidiphilum, P. phosphoreum, and P. profundum may be considered as piezophilic (former barophilic) bacteria, because these species enhance its growth at pressures above 1 atmosphere, by mean of several high-pressure adaptation mechanisms [9,74]. The adaptive traits include those related to growth, macro-molecules and storage lipids, membrane and soluble proteins, the respiratory-chain compounds, replication, transcription and translation. [9,54,90]. These species are the only ones known to produce a long-chain polynsaturated fatty acid (PUFA), the eicosapentaenoic acid (EPA) [58]. Recently, Le Bihan et al. [42] analysed the proteome of P. profundum under different pressure regimes, and obtained altered modes of protein function in that conditions. The authors identified differentially expressed proteins involved in high pressure adaptation; thus, proteins belonging to the glycolysis/gluconeogenesis pathway were up-regulated at high pressure, whils several proteins involved in the oxidative phosphorylation pathway were up-regulated at atmospheric pressure. In addition, the expression of some proteins involved in nutrient transport or assimilation was also directly regulated by pressure.
**Pathogenesis of Photobacterium**

Some species of this genus, including *P. rosenbergii*, *P. jeantii*, *P. sanctipauli*, and the two subspecies of *P. damselae*, have been reported to produce several pathologies on animal hosts, such as corals, sponges, fish, and homeothermic animals [10,56,69,83]. Unfortunately, little is known on the pathogenesis mechanisms of *P. rosenbergii* and *P. sanctipauli* that cause the coral bleaching and further dead of the corals [83]; however, both *P. damselae* subspecies have received a great attention as emerging pathogens for many aquatic organisms, including fish, mollusks and crustaceans, and even for humans [41,55,69,72,89].

*Photobacterium damselae* subsp. *damselae* (*Pdd*) is a normal inhabitant of seawater, marine sediments, seaweeds and marine animals [41,76], and prefers warm water conditions (20–30 °C). This microorganism is considered a primary pathogen of several species of wild- and cultured-fish causing wound infections and hemorrhagic septicaemia. It is also an opportunistic human pathogen, causing necrotizing fasciitis [69]. The other subspecies, *P. damselae* subsp. *piscicida* (*Pdp*), is the causal agent of fish photobacteriosis, a serious bacterial disease affecting different economically important cultured marine fish species [72].

**Virulence factors of *P. damselae***. The main bacterial iron-uptake systems include the production of iron-sequestering compounds named siderophores as well as the use of heme group as iron source. Siderophores are chemically diverse low-molecular-weight iron chelators that can effectively solubilize iron or remove it from other chelators and transport it into the cell through the corresponding membrane receptor proteins [43]. Some bacteria not only produce their own siderophores, but also express receptors capable of transport xenosiderophores produced by other organisms [11]. *Pdp* and *Pdd* are able to acquire iron from hemin and hemoglobin as unique iron sources in vitro [43]. Their heme uptake systems are encoded by a gene cluster formed by 10 genes [67]. This heme uptake system includes a TonB-dependent outer membrane receptor to transport the heme group into the periplasm, a periplasmic binding protein, and an ATP-binding cassette (ABC) to drive heme across the cytoplasmic membrane [4,67]. It is also known that in *Pdp*, the acquisition of iron from its host is efficiently achieved by means of the synthesis of the siderophore piscibactin [81], and its transport into the cell through the outer membrane receptor FrpA [62]. The synthesis and transport are encoded by a pathogenicity island, which is part of the transmissible plasmid pPHDP70. It has been demonstrated that this plasmid greatly contributes to the virulence of *Pdp* for fish, and that it can be horizontally transmitted to other marine bacteria [62]. It has also been reported that *Pdd* expresses several high-molecular-weight outer membrane proteins under iron limitation conditions [69], and that some strains likely produce the siderophore vibrioferrin [65], although other virulent strains lack this system, being its contribution to virulence yet uncertain. The presence of these or other iron uptake mechanisms in other species of *Photobacterium* is unknown, although some of the iron-uptake related genes reported in both *P. damselae* subspecies are present in other species genomes. The role of these mechanisms in non-pathogenic species is uncertain.

Bacterial extracellular products (ECP) containing phospholipase, cytotoxic, and hemolytic activities may account for the damage to infected cells, the consequent release of the microorganisms, and the invasion of adjacent cells [25]. ECP of *P. damselae* strains were shown to be lethal for different fish species and for fish and homeothermic cell lines [40]. Recently, Vences et al. [87] have demonstrated that phospholipase and collagenase activities contributed to virulence of *Pdd*. It is well known the existence of a close relationship between the ability of a microorganism to provoke diseases and the production of bacterial toxins. In the case of *Pdd*, several heat-labile cytolytic toxins have been reported, one of them named damselysin (Dly), a phospholipase-D active against sphingomyelin, presented strong hemolytic activity [38]. It has also been demonstrated that presence of gene *dly* is not a pre-requisite for the hemolytic activity and for the pathogenicity of *Pdd*, since *dly*-negative strains possess virulence potential for animals, and also show toxicity for homeotherm and poikilotherm cell lines [40,61]. Rivas et al. [68] identified and characterized a 150 kb plasmid, pPHDD1, which contains the genes for both Dly and HlyA, being the lastest a small pore-forming toxin (PFT) with hemolysin activity, named phobalysin [70]. The mutation of both *dly* and *hlyAp* genes in a pPHDD1-harbouring strain renders the strain non-virulent for fish, and only slightly virulent for mice, and the hemolytic phenotype on sheep blood agar of a *dly* and *hlyAp* double mutant resembles that of naturally plasmidless strains [68,69]. Thus, pPHDD1-harbouring isolates of *Pdd* produce three different hemolysins, each of them individually prove to be sufficient to cause death in mice. Each hemolysin contributes to virulence in a different degree, although only
the Dly-producing strains caused death in fish, demonstrating the importance of the plasmid for the virulence of this bacterium for fish. Despite the importance of pPHDD1, many Pdd virulent strains are plasmidless. The hemolytic activity exhibited by these strains is due to hemolysin PhlyC, encoded by the chromosome-harbored hlyA gene [69,87], which contributes to virulence for fish [87].

In Pdp a key pathogenicity factor is an exotoxin, a plasmid-encoded apoptosis-inducing protein of 56 kDa (AIP56), responsible for apoptotic activity against fish macrophages and neutrophils [16]. The AIP56 toxin is a zinc metalloprotease involved in binding and internalization into the cytosol of target cells [77], and acts inducing the activation of caspases 8, 9, and 3, the loss of mitochondrial membrane potential, the release of cytochrome c into the cytosol, and the overproduction of ROS, which suggest that the exotoxin activates both extrinsic and intrinsic apoptotic pathways [12]. Through the activation of the cell death process involving macrophages and neutrophils, the pathogen is able to subvert the immune defenses of the host and to produce infectious disease.

Little is known on the adherent properties to cells of Pdd, although Khouadia et al. [35] established that this subspecies possess the ability to adhere to fish mucus. On the contrary, Pdp is adherent mainly for fish cells [53], and the adherence is heat-sensitive, but it is not affected by proteases or by treating the bacteria with antisera raised against its LPS [53]. Nevertheless, the precise nature of the mechanism responsible for adherence and interaction with host cell receptors and virulence factors contributing to the invasion of fish nonphagocytic cells is still unknown [1,3].

Pdp is considered weakly to moderately invasive to several poikilothermic cell lines. López-Doriga et al. [44] showed that the uptake of Pdp by EPC cells is time and bacterial-concentration dependant. These authors have been suggested that internalization of this microorganism by EPC cells is receptor-ligand mediated (zipper mechanism). Pdp isolates also show the ability to spread to adjacent cells from initially infected cells, forming plaques of dead cells [53]. Similar to that previously reported for other Gram-negative pathogenic bacteria, invasion by Pdp can be inhibited by cytochalasin D, indicating that actin and microfilament-dependent mechanisms are required for bacterial internalization [53].

Virulent Pdp strains are serum resistant and can grow in fresh fish serum, whereas non-virulent strains are sensitive to serum killing and their growth is totally inhibited in fresh serum [3]. The inhibitory effect of the serum on the non-viral strains, however, is totally lost if the complement is inactivated by heating at 56 °C for 1 h [51]. Serum resistance is also associated with capsule production, since capsulated strains prevent formation of C3 convertase (C3bBb) by failing to bind serum protein B, or by a higher affinity for serum protein H than for B. Therefore, capsulated strains evade more efficiently the bactericidal activity of fresh serum [53]. Pdp capsule formation depends on growth conditions; thus, cells grown under iron-limited conditions or old-cultures had a significantly reduced amount of capsular material [14]. Studies that describe the contribution of bacterial capsules to adhesion and invasion of host cells are contradictory [44,52]. The presence of a capsule prevents the opsonization by C3b, and bacteria will not efficiently be engulfed by fish macrophages [5]. Furthermore, the capsule plays an important role in lethality of Pdp to fish, as non-virulent strains induced for capsule expression resulted in a reduction of LD50 values [52].

The ability of Pdp to avoid phagocytosis and thus to cause disease, may be explained by the induction of extensive apoptosis on macrophages and neutrophils present in Pdp-infected foci, resulting in lysis of these leukocytes by post-apoptotic secondary necrosis [15]. There are contradictory results on the interaction of Pdp with phagocytes; whereas intact bacteria within phagocytes have been observed in vivo [59], suggesting that Pdp may survive inside macrophages, other in vitro studies indicate that fish macrophages are able to kill the bacteria by means of activation of the respiratory burst or an iron-SOD activity [5,8,78].

Future perspectives

In this review, we have described some aspects of the genus Photobacterium, including taxonomy, phylogeny, ecology and pathological mechanisms. There is still a lack of understanding of several features encoded by Photobacterium genomes, such as the novel genes involved in the adaptation to specific habitats, the study of new metabolic pathways and their involved genes, and other cellular functions and metabolites produced by these microorganisms. Moreover, the ability of several species of this genus to produce polyunsaturated fatty acids, cold-adapted
enzymes and antimicrobial compounds constitutes new ways of investigation for a potential biotechnological application of these products in the future.

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References


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