The biotechnological potential of marine bacteria in the novel lineage of *Pseudomonas pertucinogena*

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**Summary**

Marine habitats represent a prolific source for molecules of biotechnological interest. In particular, marine bacteria have attracted attention and were successfully exploited for industrial applications. Recently, a group of *Pseudomonas* species isolated from extreme habitats or living in association with algae or sponges were clustered in the newly established *Pseudomonas pertucinogena* lineage. Remarkably for the predominantly terrestrial genus *Pseudomonas*, more than half (9) of currently 16 species within this lineage were isolated from marine or saline habitats. Unlike other *Pseudomonas* species, they seem to have in common a highly specialized metabolism. Furthermore, the marine members apparently possess the capacity to produce biomolecules of biotechnological interest (e.g., dehalogenases, polyester hydrolases, transaminases). Here, we summarize the knowledge regarding the enzymatic endowment of the marine *Pseudomonas pertucinogena* bacteria and report on a genomic analysis focusing on the presence of genes encoding esterases, dehalogenases, transaminases and secondary metabolites including carbon storage compounds.

**Introduction**

The oceans cover the largest part of the earth’s surface and represent one of the most diverse environments on our planet (Venter et al., 2004; Armbrust and Palumbi, 2015; Tully et al., 2018). Researchers have started to explore this diversity by identification and isolation of biocatalysts and secondary metabolites produced by marine organisms, opening up a novel branch of research and application termed blue biotechnology. Examples comprise not only the famous green fluorescent protein produced by the jellyfish *Aequorea victoria* and its use in innumerable applications including clinical diagnostics and therapeutics (Ohba et al., 2013; Enterina et al., 2015; Hoffman, 2015), but also antiviral and anticancer compounds isolated from marine sponges (Calcabrini et al., 2017), biocatalysts with potential application for the production of pharmaceutical building blocks like solketal (Ferrer et al., 2005), and trabectedin (supplied as Yondelis by PharmaMar S.A.) as an example for a chemotherapeutic compound produced by putative endosymbiotic bacteria of the sea squirt *Ecteinascidia turbinata* (Schofield et al., 2015). Marine metagenomics and the identification and characterization of isolated marine organisms have led to a huge set of gene and genome sequences, as recently shown by the assembly of more than 2600 draft genomes from data collected during the Tara Oceans circumnavigation expedition (Tully et al., 2018), as well as experimental data enabling insights into the biochemical potential of marine habitats and the respective microorganisms (Li and Qin, 2005; Kennedy et al., 2008; Popovic et al., 2015).

A number of marine bacteria hold great potential for biotechnological applications, for example the marine bacterium *Alcanivorax borkumensis*, which is known to play a key role in bioremediation of oil spills (Schneiker et al., 2006). The strain produces a biosurfactant (Yakimov et al., 1998) and possesses several genes...
encoding esterases and monoxygenases of high biotechnological interest (Tchigvintsev et al., 2015). Several marine Acinetobacter sp. were also shown to produce biosurfactants (Mnil and Ghribi, 2015), for example glycolipoproteins with useful surface-active properties (Peele et al., 2016). An Enterobacter species isolated from a shark jaw produces rather uncommon medium-chain-length polyhydroxyalkanoates which may have biomedical applications (Wecker et al., 2015). Furthermore, a range of novel antibiotics was identified from marine bacteria of diverse sources (Eom et al., 2013).

Members of the genus Pseudomonas which belongs to the γ-proteobacteria can colonize diverse habitats and produce useful biomolecules including lipases (Jaeger et al., 1996; Liu et al., 2017), fluorescent proteins (Torra et al., 2015), degradation pathway enzymes (Poblete-Castro et al., 2012), rhamnolipids (Chong and Li, 2017), phenazines (Bilal et al., 2017) and a number of heterologous secondary metabolites (Loeschcke and Thies, 2015). The main fraction of Pseudomonas species described so far is assigned to terrestrial habitats (Romanenko et al., 2005); however, there are also reports of species isolated from marine environments (Baumann et al., 1983), e.g. P. marincola (Romanenko et al., 2008), P. aeruginosa (Manwar et al., 2004) or P. glareae (Romanenko et al., 2015).

The huge number of Pseudomonas species was phylogenetically distributed into three lineages comprising 13 groups. One and by far the smallest of these lineages, which was only recently established, consists of a single so-called Pseudomonas pertucinogena group. A small number of newly described Pseudomonas species with remarkable properties cluster within this lineage (García-Valdés and Lalucat, 2016; Peix et al., 2018). Originally, it consisted of only two species, namely P. pertucinogena and P. denitrificans (Anzai et al., 2000). As the classification of P. denitrificans is known to be ambiguous (Doudoroff et al., 1974), it is difficult to assign respective studies to the correct genus and species; these studies are therefore not considered within this review. The original P. pertucinogena group was recently classified as a separate lineage within the genus Pseudomonas (Peix et al., 2018) comprising nine marine (including the salt lake isolate P. salina) and seven non-marine members (Table 1).

Remarkably, enzymes from these bacteria were mentioned in different studies focusing on the bioinformatics identification of novel biocatalysts relevant for biotechnology applications, although most of the relevant species or genome sequences have been described only recently. We summarize here reports on biotechnologically relevant biomolecules produced by marine specimens of the P. pertucinogena lineage and describe their respective habitats with prevailing harsh environmental conditions. Based on the findings reported, we have examined the genetic capabilities of this group of bacteria to potentially produce biotechnologically relevant enzymes including polyester hydrolases, rare dehalogenases, ß-transaminases as well as secondary metabolites and carbon storage compounds. Apparently, bacteria of the P. pertucinogena lineage have the potential to produce such biotechnologically relevant biomolecules; however, this has for each case to be experimentally validated.

The Pseudomonas pertucinogena lineage

Representatives of the genus Pseudomonas are generally equipped with a versatile metabolism that is reflected by a rather large genome with sizes ranging from 4.1 (for some P. stutzeri strains) to more than 6 Mbp (e.g. for P. syringae, P. aeruginosa, P. putida, P. protegens and P. fluorescens). Hilker et al. reported a core genome consisting of more than 4000 open reading frames after analysing the genome sequences of 20 different P. aeruginosa strains (Hilker et al., 2015). Similar dimensions were shown for P. putida; the core genome consists of at least 3386 genes and the average genome size is reported with about 6 Mbp (Udaondo et al., 2016; Lopes et al., 2018). An analysis of 76 newly isolated fluorescent Pseudomonas strains from tropical soil showed more than 5500 coding sequences per genome and an average genome size of more than 6 Mbp for the newly assembled genomes (Lopes et al., 2018). In contrast, all members of the P. pertucinogena lineage possess, as far as known, a comparably small genome of less than 4 Mbp (Table 1, column 6), coding for about 3500 genes. These bacteria were isolated from diverse habitats, for example in association with marine sponges (Romanenko et al., 2005), from the air (Azhar et al., 2017), deep-sea sediments (Wang and Sun, 2016) or heavy metal contaminated soil (Zhang et al., 2011) and they are distributed over a large geographical area (Fig. 1). Currently, only 16 species are assigned to this lineage, with about half of them attributed to marine environments (Table 1, marked with superscript f). Most of these species were first described during the last 10 years, but the lineage is likely to be further extended in the near future, e.g. by 16 metal resistant endophytic bacteria which appear to be near relatives of P. sabulinigr isolated from marshlands (Rocha et al., 2016).

Many of the marine species of the P. pertucinogena lineage are adapted to cold environments, with reported growth at temperatures below 15°C and tolerance for moderate salt concentrations, surely related to their marine living conditions. Some species were described to live in association with aquatic plants, algae or sponges. As an example for such a symbiotic relationship,
Table 1. Bacteria belonging to the *P. pertucinogena* lineage.

| Species          | Habitata | Origena | Temperature rangeb | Salinity rangeb | Accession No c | Reference d |
|------------------|----------|---------|-------------------|-----------------|----------------|-------------|
| *P. pertucinogena* | Not recorded, deposit of the ATCC | | n.d | n.d | | Kawai and Yabuichi (1975) |
| *P. bauzanensis* | Soil from an industrial site | Bozen, South Tyrol, Italy | 5–30°C | 0–10% | NZ_FOGN00000000.1, NZ_FOUA00000000.1 | Zhang et al. (2011) |
| *P. populi* | Stems of *Populus euphratica* tree | Khiyik River, China | 20–50°C | 0–5.0% | NZ_FOYD00000000.1 | Lin et al. (2013) |
| *P. oceani* | Food-waste compost | Taiwan | 4–45°C | 1–3% | | Anwar et al. (2016) |
| *P. formosensis* | Air samples in an urban environmental | Makkah, Saudi Arabia | 37°C | n.d. | LM997413.1 | Azhar et al. (2017) |
| *P. saudimassiliensis* | Activated sludge in sewage treatment | Xiamen, China | 10–45°C | 0–8% | | Lai and Shao, 2008; |
| *P. salina* | Desert sand | Xinjiang, China | 4–42°C | 0–6% | NZ_LT629736.1 | Liu et al. (2009) |
| *P. xinjiangensis* | Salt lake | Xiaochaidan, China | 4–35°C | 0–12.0% | | Zhong et al. (2015) |
| *P. aestusnigril* | Crude oil-contaminated intertidal sand samples | Spain | 18–37°C | 2–12.5% | NZ_NBYK00000000.1 | Sánchez et al. (2014), Gomila et al. (2017b) |
| *P. litoralis* | Mediterranean seawater | Spain | 15–37°C | 0–15% | NZ_LT629748.1 | Pascaul et al. (2012) |
| *P. oceanil* | Deep-sea (1350 m) | Okinawa Trough, Pacific Ocean | 4–41°C | 0–10% | NZ_PPSK00000000.1 | Wang and Sun (2016), Garcia-Valdés et al. (2018) |
| *P. pachastrellae* | Sponge *Pachastrella* | Philippine Sea | 7–41°C | 0–10% | NZ_MUBC00000000.1 | Romanenko et al. (2005), Gomila et al. (2017a) |
| *P. pelagia* | Antarctic green algae | Antarctic Ocean | 4–33°C | 0.5–8% | NZ_AROI00000000.1 | Hwang et al. (2009), Koh et al. (2013) |
| P. pelagia | CL-AP6 (type strain) | 58°N, 13°E | | | NZ_NWMT00000000.1 | |
| *P. sabulinigil* | Black beach sand | Soesogakk, Jeju Island, Korea | 4–37°C | 0–10% | NZ_LT629763.1 | Kim et al. (2009) |
| *P. salegens* | Aquatic plants of saline wetland | Gomishan saline wetland, Iran | 4–35°C | 1–10% | NZ_LT629787.1 | Amoozegar et al. (2014) |
| *P. profundil* | Deep-sea (1000 m) | Pacific Ocean, Mariana Trench | 4–40°C | 0–10% | | Sun et al. (2018) |

a. Environment from which the species was isolated (Habitat) and geographical origin of the sample (Origin) as stated in the type strain description.
b. As stated in the respective type strain description.
c. Accession numbers of GenBank/RefSeq entries for the genomes or, in cases of draft genome sequences, the accession number of the respective master entry.
d. References for original descriptions and, if applicable, genome announcements.
e. S. Thies and A. Bollinger, unpublished data.
f. Marine isolates.

*P. pelagia* is discussed to depend on its host’s protection against freezing to survive under the harsh conditions in the Antarctic Ocean, as antifreeze activity of the bacterium itself was not observed (Koh et al., 2013). Aside from the colonization of algae, plants and sponge surfaces, contaminated environments seem to be a preferred habitat for these marine bacteria, including endophytes isolated from a heavy metal accumulating plant at a contaminated salt marshland (Rocha et al., 2016) and crude oil-contamination sites (Lamendella et al., 2014 and Sánchez et al., 2014). The ability to degrade different hydrocarbons, the major constituents...
of crude oil, was proposed for *P. aestusnigri*, which possesses genes encoding phenol hydroxylase for degradation of aromatic hydrocarbons as well as an alkane-1-monoxygenase for aliphatic alkane degradation (Gomila *et al.*, 2017b).

Bacteria belonging to the genus *Pseudomonas* are generally well known for their versatile metabolism. The metabolic flexibility of Pseudomonads is reflected by their ability to use for growth a range of different carbon sources including carbohydrates, organic acids, alcohols, alkanes and most amino acids (Palleroni, 1984; Daniels *et al.*, 2010). A comprehensive phenomics analysis of *P. putida* strain DOT-T1E revealed a complex hierarchical network regulating the utilization of different carbon, nitrogen or sulfur sources (Daniels *et al.*, 2010). In contrast, members of the novel *P. pertucinogena* lineage seem to be rather limited with respect to the spectrum of utilizable carbon sources. Sánchez *et al.* described eight species of the *P. pertucinogena* group which utilize short chain carbonic acids, e.g. lactate or propionate and few amino acids, whereas they fail to utilize glucose as well as 75–80% of all tested carbon sources provided by standard phenotyping assays (Sánchez *et al.*, 2014). Undoubtedly, additional studies are needed to explore the metabolism of these bacteria in more detail. However, the currently available data suggest that strains of the *P. pertucinogena* group may constitute an exception of niche-adapted specialists within the genus *Pseudomonas*, as already suggested by the specific sites of isolation and further corroborated by their small genomes and their limited metabolic flexibility. The psychrophilic and moderate halophilic habitats indeed suggest potential for a variety of biotechnological applications of the bacteria themselves, but also their enzymes (Cavicchioli *et al.*, 2011; Cafaro *et al.*, 2013; Yin *et al.*, 2015; Danso *et al.*, 2018).

Enzymes produced by bacteria of the *P. pertucinogena* lineage have been mentioned by several studies, in particular those useful for polymer degradation and synthesis of chiral molecules (Schallmey *et al.*, 2014; Haernvall *et al.*, 2017, 2018). Enzyme coding genes were identified by mining of sequence data and subsequently expressed in heterologous hosts to prove their functionality. The results indicate that in silico mining of *P. pertucinogenae* genomes indeed constitutes a suitable strategy to assess the biotechnological potential of this currently still small group of *Pseudomonas* species. Hence, we have analysed the genomes of seven species of the marine *P. pertucinogena* lineage with respect to their capacity to produce biotechnologically relevant enzymes and compounds. In general, BLASTP (Altschul *et al.*, 1997) was applied to identify homologues of proteins reported in literature taking at least 40% identity to the query sequence and query coverage of at least 85% as lower borders for selection. However, as a number of closely related species were analysed, the sequence homology of homologous proteins was significantly higher, as stated in the respective paragraphs.
These analyses revealed a considerable number of genes encoding useful enzymes as well as the potential to synthesize secondary metabolites and storage compounds (Table 2).

Biocatalysts

Enzymes are applied for a large number of different biotechnological applications, but increasingly also as (often enantioselective) biocatalysts in synthetic organic chemistry driving the development of green and sustainable processes (Sheldon and Woodley, 2018). Here, enzymes to be obtained from bacteria of the *P. pertucinogena* lineage can significantly contribute, in particular polyester hydrolases, dehalogenases and transaminases.

Polyester hydrolases

Carboxylic ester hydrolases (EC 3.1.1.) represent an important group of biocatalysts for industrial applications in a wide range of different sectors, like the pulp and paper, the pharmaceutical and the food industry (Singh et al., 2016). They catalyse both the hydrolysis and the synthesis of esters, often with high enantioselectivity (Casas-Godoy et al., 2012). During the last decades, the degradation of polyester compounds became more and more important due to the increasing environmental pollution with non-biodegradable polyesters such as polyethyleneterephthalate (PET) (Narancic and O’Connor, 2017). As this synthetic polyester cannot be properly recycled, more than 70% of the total plastic packaging waste may ultimately enter the food chain through inadequately treated waste water, the oceans and subsequently marine micro- and macroorganisms (Wei and Zimmermann, 2017).

The potential of *P. pelagia*, a member of the *P. pertucinogena* lineage, to effectively degrade ionic phthalic acid-based polyesters was shown recently (Haernvall et al., 2017). The respective biocatalyst is a putatively secreted lipase designated as PpelaLip, which was identified by a homology guided sequence search of different extracellular hydrolases from *Pseudomonas* sp. using as a template the amino acid sequence of the *Thermobifida cellulosislytica* cutinase (Thc_Cut1), an enzyme known to efficiently hydrolyse different polyesters. After successful recombinant production and purification, the activity of the enzyme was experimentally demonstrated with different polyester substrates (Haernvall et al., 2017). In a successive study, the applicability of this biocatalyst for wastewater treatment was shown (Haernvall et al., 2018). We also performed a homology search with BLASTP (Altschul et al., 1997) using *P. pelagia* lipase PpelaLip as a query against all published genome sequences from the *P. pertucinogena* lineage (Table 1, column 6) and identified putative proteins with 70–80% sequence identity. Interestingly, these PpelaLip homologous enzymes are not unique within the marine bacteria of the *P. pertucinogena* lineage, as we have identified homologs in all *P. pertucinogena* species (Tables 2 and 3). Apparently, representative species of the *P. aeruginosa* and *P. fluorescens* lineages also encode such enzymes; however, the overall similarity is low indicating that this type of putative polymer-degrading enzyme represents a distinct characteristic of the *P. pertucinogena* bacteria (Table 3). In a recent study of a PETase from *Ideonella sakaiensis* (Yoshida et al., 2016), the classification of PETases in three groups was suggested based on amino acid sequence alignments and putative enzymes from *P. sabulinigri*, *P. pachastrellae* and *P. litoralis* were grouped into type IIa of PET-degrading enzymes (Joo et al., 2018).

Dehalogenases

Dehalogenases catalyse the cleavage of carbon–halogen bonds and have potential applications in the chemical industry and for detoxification (Kurihara and Esaki, 2008). They are used for organic synthesis of optically pure building blocks, recycling of by-products from chemical processes, bioremediation and biosensing of toxic pollutants (Koudelakova et al., 2013). Halohydrin dehalogenases (HHDH) represent a particularly interesting type of dehalogenases which naturally catalyse the dehalogenation of haloalcohols with the formation of the corresponding epoxides. In the reverse reaction, i.e. opening of the epoxide ring, they behave promiscuous accepting a wide range of nucleophiles such as azide, cyanide or nitrite enabling the synthesis of a wide range of chiral molecules (Schallmein et al., 2014; Koopmeiners et al., 2016). Schallmein et al. recently developed a bioinformatics pipeline to uncover these rare enzymes within sequence data sets and successfully accessed selected hits by heterologous expression and subsequent demonstration of HHDH activity. During this study, an HHDH was identified in the *P. pelagia* CL-AP6 genome and assigned to subgroup D, designated as HheD12 (Schallmein et al., 2014). This same study unveiled that other *Pseudomonas* species did not encode HHDHs; and only five of 43 reported enzymes originate from γ-proteobacteria. A BLASTP analysis of the available genome data within the *P. pertucinogena* lineage with the *P. pelagia* CL-AP6 enzyme (WP_022962804.1) as a query revealed hits with identities between 91% (*P. pelagia* strain 58) and 68% (*P. salegens*) in all marine species with the exception of *P. litoralis* (Table 2). Within the terrestrial isolates, only the *P. xinjiangensis* genome encoded such an enzyme.
**Table 2.** The catalytic and biosynthetic potential of marine *P. pertucinogena* bacteria. Genome sequences were analysed with different bioinformatics tools for the presence of polyester hydrolases (PE hydrolase), halohydrin dehalogenases (HHDH and HheD12), ω-transaminases (ω-TA), flavin-binding fluorescent proteins (FbFP), polyhydroxyalkanoates (PHA) and ectoin synthesis clusters (Ectoin). 

| Species         | Strain  | PE hydrolasea | HHDHb | ω-TAc | FbFPd | PHAe | Ectoinef |
|-----------------|---------|---------------|--------|-------|-------|------|----------|
| *P. aequorii*   | VXO14   | WP_088276085.1| WP_088273591.1 | WP_088276225.1 | WP_088273209.1 | Yes  | Yes      |
| *P. litoralis*  | 2SM5    | WP_090272969.1 | –      | –     | WP_090274676.1 | –    | No       |
| *P. pachastrellae* | CCUG 46540 | WP_083724990.1 | WP_083723433.1 | WP_083728130.1 | WP_083728464.1 | Yes  | Yes      |
| *P. pelagia*    | 58      | WP_096345769.1 | WP_096348266.1 | WP_096345677.1 | WP_096346382.1 | Yes  | Yes      |
| *P. sabulinigri*| CL-AP6  | WP_022964382.1 | WP_022962804.1 | WP_022961575.1 | WP_022964449.1 | Yes  | Yes      |
| *P. salegens*   | JCM 14963 | WP_092287377.1 | WP_092284942.1 | WP_092286338.1 | WP_092288528.1 | Yes  | Yes      |
| *P. oceanii*    | CECT 8338 | WP_092388080.1 | WP_092387787.1 | WP_092388656.1 | WP_092386384.1 | Yes  | Yes      |
| *P. oceanii*    | DSM 100277 | WP_104736494.1 | WP_104737909.1 | WP_104738025.1 | WP_104739004.1 | Yes  | Yes      |

a. Proteins with at least 70% identity to the polyester hydrolase PpelaLip from *P. pelagia* (Haemvall et al., 2017).  
b. Proteins with high similarity to *P. pelagia* HheD12 (Schallmey et al., 2014).  
c. Proteins with at least 40% identity to selected known ω-TAs as query sequence and a query coverage of at least 90%.  
d. Proteins with identities >60% to PpSB1-LOV (NP_746738.1), identified by BLASTP (Altschul et al., 1997).  
e. Presence of a complete metabolite synthesis cluster predicted by the antiSMASH pipeline (Weber et al., 2015; Blin et al., 2017).  
f. Marine isolates.

This suggests an important role for these enzymes especially in marine environments.

**ω-Transaminases**

Chiral amines are valuable building blocks for a variety of compounds produced by the chemical and pharmaceutical industries. For pharmaceuticals, an estimated share of 40% contains at least one amine functionality (Kelly et al., 2018). While several options for enzymatic chiral amine production exist, asymmetric synthesis by ω-transaminases (ω-TA) is greatly preferred as the theoretical yield is 100% (Cassinjme et al., 2010; Koszelewski et al., 2010). Most TAs need pyridoxal-5’-phosphate as a cofactor and catalysate the asymmetric synthesis of chiral amines by transferring an amino group from an aminated donor to various carbonyl compounds (Savile et al., 2010; Börner et al., 2017; Guo and Berglund, 2017). A diverse array of reactions is reported towards the synthesis of pharmaceuticals or pharmaceutical intermediates involving ω-TAs by both asymmetric synthesis and kinetic resolution (Kelly et al., 2018). Thus, the identification of novel enzymes with ω-transaminase activity is of high importance for both science and industry.

*Pseudomonas* sp. appear to be a promising source for ω-transaminases (Wilding et al., 2015; Poehlein et al., 2017; Wu et al., 2017). Recently, an ω-transaminase which most probably originates from *P. sabulinigri* was discovered during an activity-based screening of a metagenomic library which originated from a polluted harbour site in Ancona, Italy (M. Ferrer, personal communication). While *P. sabulinigri* was originally identified in samples from Korean beach (Table 1), another report on closely related specimen discovered in a metalloid polluted salt marsh in the northwest coast of Portugal (Rocha et al., 2016) hints at the potential of this *Pseudomonas* strain to thrive in contaminated environments.

Based on this finding, a homology search with the BLASTP tool was performed to gain insights into the potential of marine representatives of the *P. pertucinogena* lineage for the production of ω-transaminases following a strategy reported earlier (Mathew and Yun, 2012). After searching the genome sequences of *P. aequorii*, *P. litoralis*, *P. sabulinigri*, *P. salegens*, *P. pelagia* strain 58, *P. pelagia* strain CL-AP6, *P. pachastrellae* and *P. oceanii*, the results were filtered for hits with at least 40% identity to the query sequence and query coverage of at least 90%.

The amino acid sequences of the four recently characterized (S)-selective ω-transaminases from *P. putida* (Wu et al., 2017) were used as the main set of queries. The respective enzymes belong to differing families of transaminases, namely the 4-aminobutyrate pyruvate aminotransferase family (EC 2.6.1.96, BAN53958.1), beta-alanine pyruvate transaminase family (EC 2.6.1.18, BAN5222.1), aspartate aminotransferase family (EC 2.6.1.1, BAN54951.1) and putrescine-pyruvate aminotransferase (EC 2.6.1.113, BAN57107.1), BAN5222.1,
which is identical to the enzyme used for resolving a crystal structure (PDB 3ABU), delivered one hit with P. litoralis, P. sabulinigri, P. salegens, P. pelagia (both strains) and P. pachastrellae, and two hits with P. aequusnigri and P. oceani. Additional searches with sequences of BANS3958.1, BAN5495.1 and BAN57107.1 each returned the same enzyme sequence in every examined genome, as well as a second hit in the genome of P. pelagia CL-AP6. All hits have been annotated as aspartate aminotransferase family proteins according to the NCBI database.

Additional homology searches with sequences of (S)-selective \( \omega \)-transaminases from Ruegeria pomeroyi, Vibrio fluvialis and Chromobacterium violaceum, all attributed to the aspartate aminotransferase family, returned hits with the same amino acid sequences as the aforementioned (S)-selective transaminases from P. putida, with identities up to 60%. Homology searches with (R)-selective transaminases from Aspergillus fumigatus, Aspergillus fisheri, Fusarium graminearum and Arthrobacter sp. according to Pavlidis et al. (2016) delivered no results within the search parameters. Conclusively, every searched genome contains at least two structurally different putative \( \omega \)-transaminases, P. aequusnigri, P. oceani and P. pelagia CL-AP6 contain three such candidate enzymes (Table 2).

**Flavin-binding fluorescent proteins**

Flavin-binding fluorescent proteins (FbFPs) were developed as reporter proteins which constitute an oxygen-independent alternative to the family of green fluorescent proteins. They are derived from blue light photoreceptors of the L(light)-O(oxygen)-V(oltage) domain family (Drepper et al., 2013; Buckley et al., 2015). Besides their \( O_2 \) independence, FbFPs are small proteins (\( M_r \): 12–16 kDa) and exhibit fast folding kinetics; thus, they are valuable reporter proteins for quantitative real-time analysis of different bio(techno)logic processes (Potzke et al., 2012; Rupprecht et al., 2017). Commonly, LOV photoreceptors consist of LOV domains fused to various effector domains which are activated by conformational changes of the LOV domain in response to a light stimulus. However, predominantly among bacteria, the so-called short LOV proteins only consisting of the light-perceiving LOV receptor domain have been identified (Losi and Gartner, 2008) with PpSB1-LOV originating from P. putida representing a well-studied example (Drepper et al., 2007; Wingen et al., 2014). As it is known that marine environments are a rich source for LOV proteins (Pathak et al., 2012), we investigated the genomes of the marine P. pertucinogena bacteria for occurrence of short LOV proteins using a BLASTP search for homologues of PpSB1-LOV (NP_746738.1). We identified proteins with >63% identity in every marine strain with exception of P. litoralis. The highest identity observed was 75% for a P. aequusnigri protein. While it is reported that short LOV proteins occur in 10% of all *Pseudomonas* species generally (Rani et al., 2013), this seems to be a remarkable frequency. Hence, the *P. pertucinogena* lineage may represent a promising source for novel FbFPs with unique properties given the fact that these bacteria can thrive in dark, cold and toxic environments.

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**Table 3. Amino acid sequence homology expressed as identity in percentage to the known polyester hydrolase PpelaLip (Haernvall et al., 2017) from *P. pelagia* strain CLAP6 as identified using BLASTP (Altschul et al., 1997).**

| No. | Organism                      | Strain | Identity in % | Protein ID       |
|-----|-------------------------------|--------|---------------|-----------------|
| 1   | *P. pelagia*                  | CL-AP6 | 100           | WP_02964382     |
| 2   | *P. pelagia*                  | 58     | 80            | WP_066345769    |
| 3   | *P. aequusnigri*              | VGXO14 | 74            | WP_088276085    |
| 4   | *P. litoralis*                | 2SM5   | 73            | WP_090272969    |
| 5   | *P. pachastrellae*            | JCM 12285 | 74   | WP_083724990    |
| 6   | *P. sabulinigri*              | JCM 14963 | 72   | WP_092287377    |
| 7   | *P. sabulinigri*              | JCM 14963 | 71   | WP_092287378    |
| 8   | *P. salegens*                 | CECT 8338 | 72   | WP_092388080    |
| 9   | *P. salegens*                 | CECT 8338 | 70   | WP_092388077    |
| 10  | *P. oceani*                   | DSM 100277 | 74   | WP_104736494    |
| 11  | *P. formosensis*              | JCM 18415 | 73   | WP_090538641    |
| 12  | *P. saudiamassiliensis*       | 12M76   | 73            | WP_044499735    |
| 13  | *P. xinjiangensis*            | NRRL B-51270 | 72 | SDS099569       |
| 14  | *P. xinjiangensis*            | NRRL B-51270 | 76 | WP_093397383    |
| 15  | *P. syringae*                 | ICMP19850 | 31   | KPW53696        |
| 16  | *P. aeruginosa*               | PA01    | 28            | WP_00143191     |
| 17  | *P. putida*                   | ATH-43   | 27            | WP_046786320    |
| 18  | *P. protegens*                | 4       | 24            | WP_102863500    |
| 19  | *P. stutzeri*                 | 28a39   | 52            | WP_102852227    |
| 20  | *P. fluorescens*              | C3      | 29            | WP_046049461    |
Secondary metabolites and storage compounds

*Pseudomonas* species, in general, produce a number of well-studied secondary metabolites, for example, rhamnolipids, phenazines, pyoverdines or syringafactins (Laursen and Nielsen, 2004; Visca et al., 2007; Burch et al., 2014; Tiso et al., 2017). In contrast, the secondary metabolism of the *P. pertucinogena* members still remains undiscovered, despite the eponymous pertucin produced by *P. pertucinogena* that was described to be active against phase 1 *Bordetella pertussis* (Kawai and Yabuichi, 1975). We therefore mined the genome data available via Genbank (Table 1, column 6) of the marine *P. pertucinogena* lineage organisms with respect to secondary metabolite production pathways applying the antiSMASH pipeline with enabled cluster finder algorithm (Weber et al., 2015; Bin et al., 2017). Not surprising, large gene clusters encoding modular polyketide or non-ribosomal peptide synthetases (NRPS) were very rarely detected within these comparably small-sized genomes. Only in the genome sequences of *P. sabulinigri*, an NRPS cluster was predicted that contains four adenylation domains suggesting the synthesis of a hitherto undescribed tetrapeptide derivative. Furthermore, *P. sabulinigri* as well as *P. salegens* and *P. pelagia* strain 58 possess putative biosynthesis pathways for aryl-polyenes, a widespread class of antioxidant natural products (Schöner et al., 2016), which, however, seems to be of less importance for biotechnological applications.

The aforementioned capability of *P. pertucinogena* for pertucin production suggests that bacteriocin production might also be found as a feature of these bacteria. Bacteriocins are ribosomally produced peptides which are post-translationally processed to become antimicrobial peptides and may thus be applied by the pharma or food industries (Hassan et al., 2014; Yang et al., 2014). Bacteriocin clusters were indeed predicted in the genomes of several species (*P. sabulinigri*, *P. aequusnigri*, *P. pachastrellae*, *P. pelagia* CL-AP6, *P. oceani*), but they do not appear as a common feature.

In contrast, gene clusters coding for the biosynthesis of the osmoprotectant ectoin are common among the *P. pertucinogena* bacteria and were predicted in all genomes, regardless of whether marine or soil origin. Ectoines are biotechnologically produced, e.g. with *Halomonas* spec., and used as moisturizing ingredients in cosmetics (Yin et al., 2015; Bownik and Stepniewska, 2016). Ectoin synthesis is widespread among marine or halophilic bacteria (Yin et al., 2015) as it helps to cope with high salt concentrations allowing growth at salt concentrations up to 8%, and in some cases even 15%, as reported for *P. pertucinogena* lineage bacteria (Table 1, column 5).

Notably, several of the investigated genomes seem to contain elements similar to the emulsan biosynthetic pathways. Emulsan is a surface-active polymeric bioemulsifier best known from *Acinetobacter* species. Bioemulsifiers and biosurfactants are considered as interesting natural products for biotechnological applications as detergents or emulsifiers in consumer products, pharmaceutical or environmental applications (Rosenberg and Ron, 1997; Fracchia et al., 2014; Gudina et al., 2016). Biosurfactant or bioemulsifier production would fit the observed surface activity in cultures of *P. pachastrellae* (Antoniou et al., 2015) and is furthermore known among bacteria living in oil-contaminated environments (Satpute et al., 2010; Cafaro et al., 2013). A MultiGeneBlast using the *Acinetobacter iwofii* emulsan cluster (Acc. No. AJ243431.1) as input sequence (Medema et al., 2013) revealed that none of the marine strains contains a complete cluster; furthermore, all species lack a protein homologous to the respective polymerizing enzyme Wzy. These strains also lack lipopeptide-related non-ribosomal peptide synthetase (NRPS) clusters as well as operons with homology to the rhamnolipid synthesis genes *rhlAB* from *P. aeruginosa*; hence, the biosurfactant production capacities of the *P. pertucinogena* bacteria remain undiscovered.

Bacterial carbon storage compounds, namely triacylglycerols or wax esters and polyhydroxyalkanoates (PHA), are also of interest for biotechnology (Alvarez and Steinbüchel, 2002; Steinbüchel and Lütke-Eversloh, 2003) with the latter compounds discussed as a naturally produced alternative to common petroleum-derived polyester materials (Naranic and O’Connor, 2017). Gene loci encoding for PHA production are present in all available genomes of the marine *P. pertucinogena* bacteria according to a MultiGeneBlast analysis using the PHA locus of *P. putida* KT2440 from AE015451 as input sequence. This result confirms microscopical observations of PHA granules within the cells reported for some of the species (Liu et al., 2009). Remarkably, no homologous gene clusters were identified in all terrestrial species tested. Noteworthy, all marine species contained homologs to the wax ester or triacylglyceride synthases of *Alcanivorax borkumensis* and *Marinobacter hydrocarbonoclasticus* (Alvarez, 2016). Marine *P. pertucinogena* species may thus be able to adapt their carbon storage metabolite production to the respective environmental conditions.

Conclusions

Bacterial species belonging to the recently established *P. pertucinogena* lineage are barely explored until today; nevertheless, it appears that they clearly diverge from other *Pseudomonas* species with respect
to their metabolism, genome size and, not least, environmental conditions. Notably, by applying bioinformatics tools for genome mining, we discovered that these bacteria hold a high potential for a variety of biotechnological applications. Presumably, these findings will be corroborated by further approaches of whole genome sequencing, in silico genome data mining, gene synthesis and expression in established hosts, which will further expand the still limited set of enzymes from already reported but also from other relevant enzyme classes, e.g. keto-reductases. In addition, the bacteria themselves may increasingly be used for biotechnological applications, in particular, psychro- and halophilic as well as hydrocarbonoclastic and heavy metal tolerant bacteria (Margesin and Feller, 2010; Cavicchioli et al., 2011; Cafaro et al., 2013; Yin et al., 2015). The current reports on characterized enzymes are limited to only a few marine species, but it is tempting to speculate that future studies on terrestrial species of the P. pertucinogena lineage may uncover such features as well; especially in species as P. bauzanensis which can cope with toxic contaminations. P. pertucinogena bacteria are easy to cultivate, at least in complex media, and can biosynthesize natural products such as PHAs, ectoin or bioemulsifiers, even with hydrocarbon pollutions or human-made polymers as alternative carbon sources, thus contributing to the saving of natural resources (Wierckx et al., 2015).

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Conflict of interest

None declared.

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