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REVIEW

Bioactive substances produced by marine isolates of *Pseudomonas*

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Abstract *Pseudomonas* is a genus of non-fermentative gram-negative *Gammaproteobacteria* found both on land and in the water. Many terrestrial isolates of this genus have been studied extensively. While many produce bioactive substances, enzymes, and biosurfactants, other *Pseudomonas* isolates are used for biological control of plant diseases and bioremediation. In contrast, only a few marine isolates of this genus have been described that produce novel bioactive substances. The chemical structures of the bioactive substances from marine *Pseudomonas* are diverse, including pyroles, pseudopeptide pyrrolidinedione, phloroglucinol, phenazine, benzaldehyde, quinoline, quinolone, phenanthren, phthalate, andrimid, moiramides, zafrin and bushrin. Some of these bioactive compounds are antimicrobial agents, and dibutyl phthalate and di-(2-ethylhexyl) phthalate have been reported to be cathepsin B inhibitors. In addition to being heterogeneous in terms of their structures, the antibacterial substances produced by *Pseudomonas* also have diverse mechanisms of action: some affect the bacterial cell membrane, causing bacterial cell lysis, whereas others act as acetyl-CoA carboxylase and nitrous oxide synthesis inhibitors. Marine *Pseudomonas* spp. have been isolated from a wide range of marine environments and are a potential untapped source for medically relevant bioactive substances.

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Introduction

Pseudomonas is a genus of non-fermentative gram-negative *Proteobacteria* that have been isolated from soil and fresh and salt water. This genus consists of species that are diverse genetically [5, 41, 50]. Based on their rRNA–DNA similarity, members of the genus *Pseudomonas* have been divided into five groups. Group I includes the γ subclass of the *Proteobacteria*, i.e., the genus *Pseudomonas* (sensu stricto) [53]. The species in the other four groups have been classified according to the new taxonomy [41]. Anzai et al. [5] subdivided *Pseudomonas* (sensu stricto) into two clusters based on an analysis of their 16S rDNA sequences. The first cluster has six groups: *P. syringae* (12 species), *P. chlororaphis* (5 species), *P. fluorescens* (18 species), *P. putida* (six species), *P. aeruginosa* (11 species), and *P. stutzeri* (3 species). The second cluster has only one group, the *P. pertucinogena* group (two species).

As many as 3,800 biologically active microbial metabolites have been isolated from bacteria. *Pseudomonas* produces 795 bioactive substances, including 610 antibiotics and 185 substances with bioactive properties other than antibiotic activity [18]. More bioactive substances have been isolated from terrestrial *Pseudomonas* than from marine *Pseudomonas*, and there are a vast number of terrestrial *Pseudomonas* species that are well known as producers of bioactive substances. Some of these play very important roles in the biological control of pathogenic plant bacteria and fungi and in bioremediation. In contrast, there are many

fewer isolates of marine *Pseudomonas*, and their bioactive substances are not as well studied.

Some reports indicate that there are marine and terrestrial *Pseudomonas* isolates that produce the same secondary metabolites, but novel secondary metabolites from marine isolates of this genus also have been described. The marine habitat has enormous ecological diversity, making it likely that marine *Pseudomonas* sp. represent an untapped reservoir of novel bioactive substances. This review provides a comprehensive picture of what is currently known about bioactive substances produced by marine isolates of *Pseudomonas*, their biological activities and modes of action, the ecological diversity of marine *Pseudomonas*, and the phylogenetic relationship among marine *Pseudomonas* isolates.

Bioactivities of *Pseudomonas* metabolites

Antimicrobial, antiviral, and cytotoxic agents

The first marine isolate of *Pseudomonas* known to produce a bioactive substance was isolated from *Thalassia* (turtle grass) near La Parguera, Puerto Rico [17]. The bacterium, *P. bromoutilis*, produces a pyrrole antibiotic, 2,3,4-tribromo-5(1′hydroxy,2′,4′-dibromophenyl) pyrrole. This pyrrole antibiotic inhibits gram-positive bacteria such as *Staphylococcus aureus*, *Diplococcus pneumoniae*, and *Streptococcus pyogenes* at concentrations of 0.0063 μg/ml, and inhibits *Mycobacterium tuberculosis* at 0.2 μg/ml. It is inactive against gram-negative bacteria and the fungus *Candida albicans*. This substance was not toxic in mice at 25 or 250 mg/kg by intravenous and subcutaneous injection, respectively. However, subcutaneous injection of 200 mg/kg of this pyrrole antibiotic did not protect mice infected with *S. aureus*. This was the first effort to explore the bioactivity of a substance from a marine isolate of *Pseudomonas*.

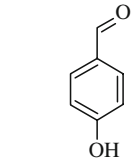
Wratten et al. [78] isolated the antibiotic-producing *Pseudomonas* sp. 102-3 from a seawater sample from a La Jolla, California tide pool. The bacterium inhibits the growth of *Vibrio anguillarum*, *V. harveyi, S. aureus*, and *C. albicans*, and produces three antibacterial compounds: 4-hydroxybenzaldehyde, 2-*n*-heptyl-4-quinolinol, and 2-*n*-pentyl-4-quinolinol (Fig. 1). 4-hydroxybenzaldehyde exhibits low antimicrobial activity, inhibiting *C. albicans*, *S. aureus*, and *V. harveyi* at 5 mg/disk, but not at 50 μg/disk. The other two compounds, 2-*n*-heptyl-4-quinolinol and 2-*n*-pentyl-4-quinolinol, both show antimicrobial activity against *S. aureus*, *V. harveyi*, and *V. anguillarum* at 50 μg/disk. As 2-*n*-heptyl-4-quinolinol is a known antibiotic that has been isolated from terrestrial *P. aeruginosa*, Wratten et al. [78] suggested that *Pseudomonas* sp. 102-3 may thus have the same biosynthetic capabilities as *P. aeruginosa*.

The marine isolates *P. fluorescens*, isolated from the surface of tunicates, have been reported to produce three unique substances, moiramides A, B, and C, along with a known compound, andrimid (Fig. 1); the latter is a member of a newer class of antibiotics, the pseudopeptide pyrrolidinedione antibiotics [51]. In contrast to moiramides A and C, moiramide B and andrimid both have antibacterial activity. This suggests that the intact succinimide moiety is required for their activity. Moiramide B and andrimid have broad-spectrum antibacterial activity because of their inhibition of bacterial acetyl-CoA carboxylase [26]. Moiramide B is the first potent antibacterial compound from marine bacteria with acetyl-CoA carboxylase inhibiting activity as its mode of action. Freiberg et al. [27] evaluated the in vivo efficacy of moiramide B and some of its synthetic derivatives using a *S. aureus* sepsis model in mice. These pyrrolidinedione derivatives exhibit antibacterial activity with minimum inhibitory concentrations (MICs) of 0.01–8, 0.25–32, and 16–64 μg/ml against *S. aureus* 133, *S. pneumoniae* G9A, and *Escherichia coli* Neumann, respectively. When evaluated in a murine model of *S. aureus* sepsis, two of the moiramide B derivatives also showed in vivo activity comparable to linezolid, an antibiotic that is used currently. These reports indicate that antibiotics produced by marine isolates of *P. fluorescence* may be potential lead compounds in the search for new classes of antibiotics to treat bacterial infections.

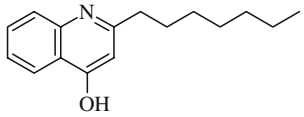
Pseudomonas sp. 1531-E7 isolated from a sponge, *Homophymia* sp., produces quinolones (2-undecyl-4-quinolone, 2-undecen-18-yl-4-quinolone, 2-nonyl-4-quinolone, and 2-nonyl-4-hydroxyquinoline *N*-oxide) (Fig. 1) [16]. Anti-*Plasmodium falsifarum* activity is exhibited by 2-undecyl-4-quinolone, 2-undecen-18-yl-4-quinolone, and 2-nonyl-4-quinolone. Cytotoxicity to KB cells is noticed for 2-undecen-18-yl-4-quinolone and 2-nonyl-4-hydroxyquinoline *N*-oxide. In addition, 2-undecyl-4-quinolone and 2-nonyl-4-hydroxyquinoline *N*-oxide are active against HIV-1 and *S. aureus*.

Isnansetyo et al. [35] purified the antibiotic 2,4-diacetylphloroglucinol (DAPG) from the culture supernatant of *Pseudomonas* sp. AMSN, which was isolated from a marine alga. The antibiotic exhibits potent activity against ten clinical isolates of methicillin-resistant *S. aureus* (MRSA), with an MIC range of 0.25–1 μg/ml, and has bactericidal activity against MRSA at 4 μg/ml, comparable to vancomycin. This antibiotic causes lysis of MRSA and *V. parahaemolyticus* at 1 and 24 μg/ml, respectively [38]. DAPG was also active against 23 vancomycin-resistant *S. aureus* (VRSA) strains isolated in Asia, Europe, Brazil, South Africa, and the USA, and against vancomycin-resistant *Enterococcus* spp. (VRE) genotypes A and B [34]. DAPG is active against a wide range of VRSA isolates as well as against a vancomycin-heteroresistant *S. aureus*

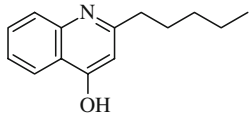
Fig. 1 The chemical structures of secondary metabolites produced by marine isolates of *Pseudomonas*. Please see the text for references



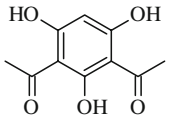
4-hydroxybenzaldehyde



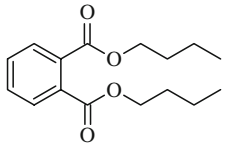
2-heptylquinolin-4-ol



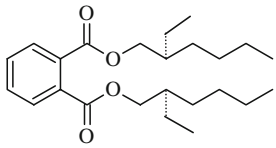
2-pentylquinolin-4-ol



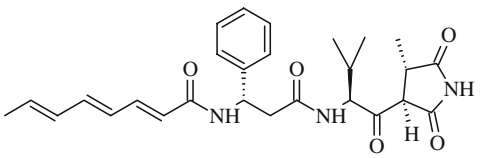
diacetyl phloroglucinol



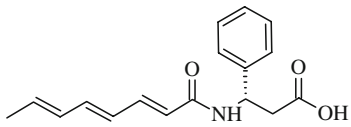
dibutyl phthalate



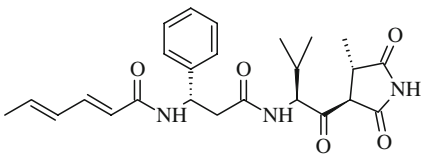
di-(2-ethylhexyl)phthalate



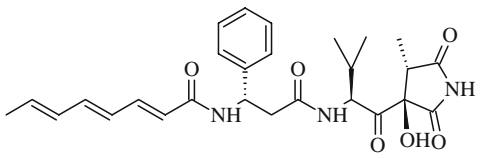
Andrimid



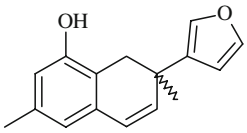
Moiramide A



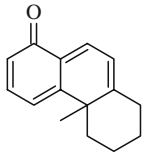
Moiramide B



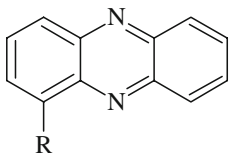
Moiramide C



Bushrin



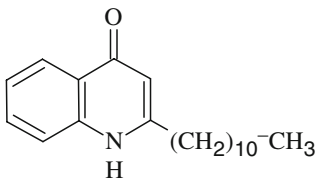
Zafrin



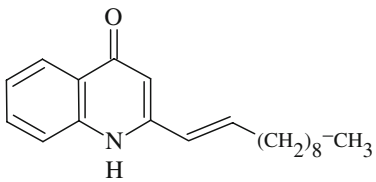
phenazine alkaloids

(1) R=-CO_2H

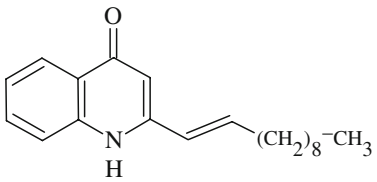
(2) R=-$CONH_2$



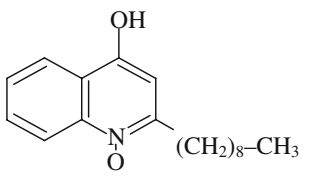
2-undecyl-4-quinolone



2-undecen-1′-yl-4-quinolone



2-nonyl-4-quinolone



2-nonyl-4-hydroxyquinoline *N*-oxide

(h-VRSA) at MIC 4 μg/ml. This substance also has moderate activity against both VRE-A and -B at MIC 8 μg/ml, but is inactive against VRE-C at up to 16 μg/ml. DAPG is a well-known antibiotic that is also produced by terrestrial *P. fluorescens* [52, 66, 79]; this suggests that AMSN has the same antibiotic biosynthesis capabilities as *P. fluorescens*.

P. aeruginosa isolated from an antarctic sponge, *Isodictya setifera*, produces six diketopiperazines and two phenazine alkaloids (Fig. 1) [37]. Phenazines are pigments with antibiotic properties. These two phenazine alkaloids are active against gram-positive bacteria, *Bacillus cereus, Micrococcus luteus*, and *S. aureus*, but diketopiperazines do not exhibit antibiotic and cytotoxic properties. *P. aeruginosa,* isolated from a mangrove environment, also produces phenazines [63]. The authors identified these pigments as pyocyanin and pyorubrin. These compounds exhibit antibacterial activity against *Citrobacter* sp. as well as hemolytic activity in a chick blood assay. Phenazine is commonly produced by terrestrial fluorescent *Pseudomonas* isolates [46], including terrestrial *P. aeruginosa.* These findings further support the idea that some marine *Pseudomonas* isolates have the same biosynthetic capabilities as their terrestrial counterparts.

A sulfated polysaccharide produced by *Pseudomonas* sp. WAK-1 isolated from the brown seaweed *Undaria pinnatifida* is active against herpes simplex virus 1 (HSV-1) (EC_{50} = 1.4 μg/ml) [47]. The polysaccharide has a repeating unit: -2)-b-d-Galp(4SO4)(1-4)[b-d-Glcp(1-6)]-b-d-Galp(3SO4)(1- [48]. Furthermore, anti-cancer activity of this substance is also determined by the panel of 39 cell lines with 63.2 μg/ml of average IC_{50}. Apoptosis is responsible for its anti-cancer activity.

Of the marine isolates of *Pseudomonas, P. stutzeri* have been described in the greatest detail. However, few studies have focused on bioactive substances produced by *P. stutzeri.* Uzair et al. [77] reported that the marine *Pseudomonas* sp. CMG1030 has antimicrobial activity. This organism was originally identified as *P. aeruginosa*, but the strain identification was revised to *P. stutzeri* CMG1030, which produces the novel antibacterial compound zafrin [76]. Subsequently, the authors elucidated the chemical structure of zafrin and studied its antibacterial mechanism. Zafrin, [4b-methyl-5,6,7,8 tetrahydro-1 (4b-H)-phenanthrenone] (Fig. 1) is active against the human pathogenic bacteria, *S. aureus* and *S. typhi*, but is inactive against *C. albicans* and *Schizosaccharomyces pombe*. The MICs for gram-positive and gram-negative bacteria range from 50 to 75 and 75 to 125 μg/ml, respectively. Further study of the mechanism of zafrin revealed that the substance exerts its bactericidal effects by bacterial cell lysis.

Another novel antibiotic, bushrin (7-(3-furyl)-3,7-dimethyl-7,8-dihydro-1-naphthalenol), is also produced by *P. stutzeri* CMG1030 [1]. This antibiotic is active against *V. harveyi, E. coli, Salmonella typhi, Proteus mirabilis, P. morgani, Shewanella putrifaciens, Hafnia alvei, Seratia marcescens, B. subtillis, B. steriothermophilus, B. cereus, Clostridium perfringens, C. sporogenes, S. aureus* (MSSA and MRSA), *S. epidermidis, E. faecalis*, and *E. faecium* with MIC ranging from 50 to 125 μg/ml. However, this antibiotic is inactive against *C. albicans, Klebsiella aerogenes*, and *Streptococcus* group G. Bacterial cell lysis activity is responsible for the antibacterial activity of this substance.

There are several marine *Pseudomonas* sp. with antimicrobial activity that is due to substances that have not yet been isolated or characterized. For example, marine *Pseudomonas* strain I-2, isolated from estuary water, has anti-*Vibrio* activity [20]. The antibacterial activity was evaluated against the following shrimp pathogenic vibrios: *V. harveyi*, *V. fluvialis*, *V. parahaemolyticus*, *V. damsela,* and *V. vulnificus*. The active substance is a non-proteinaceous substance that is soluble in chloroform. The chloroform extract from the bacterium is active against *V. harveyi* at 20 μg/ml, but there is no toxic effect on shrimp larvae up to 50 μg/ml. This suggests that the substance can be used to control pathogenic marine *Vibrio*. As *Pseudomonas* sp. I-2 is non-pathogenic to shrimp larvae, the bacterium may be used as a biocontrol agent against vibriosis in marine aquaculture.

The extract of *Pseudomonas* sp. PB2 associated with a sponge, *Suberites domuncula*, exhibits anti-angiogenic, hemolytic, antimicrobial, and cytotoxic activities [71]. Its anti-angiogenic activity in the chick chorio-allantoic membrane (CAM) assay is 50% at 5 μg/ml and 100% at 10 μg/ml. Furthermore, the extract is also cytotoxic to PC12 and HeLa cells. Antimicrobial activity of the extract is noticeable against *S. aureus*, *S. epidermidis*, *S. lentus*, *E. coli*, and *Candida albicans*.

Kim et al. [43] reported *P. fluorescens* HAK-13, which has algal-lytic activity against *Heterosigma akashiwo* (Raphidophyceae), *Alexandrium tamarense,* and *Cochlodinium polykrikoides*, but is inactive against *Gymnodinium catenatum*. The substance responsible for the activity is a proteinaceous compound that localizes to the cytoplasmic membrane of the bacterium. *P. fluorescens* HAK-13 thus has potential for selectively controlling harmful algal blooms in the marine environment.

Radjasa et al. [55] isolated *Pseudomonas* sp. associated with the soft coral *Sinularia polydactyla*. This bacterium has antibacterial activity against *Streptococcus equi* subsp. zooepidemics. Although the nonribosomal peptide synthetase (NRPS) gene from this bacterium can be amplified, the substance responsible for the antibacterial activity remains unknown. The authors have not determined the relationship between the NRPS gene and the antibacterial activity of the *Pseudomonas* sp.

Cathepsin B inhibitors

Hoang et al. [32] isolated two cathepsin B inhibitors from the culture supernatant of marine *Pseudomonas* sp. PB01. The inhibitors were identified as dibutyl phthalate and di-(2-ethylhexyl) phthalate, which exhibit dose-dependent cathepsin B with IC_{50}s of 0.42 and 0.38 mM, respectively. The substances inactivate the pericellular cathepsin B of murine melanoma cells. Cathepsin B (EC3.4.22.1), which belongs to the papain superfamily, is a cysteine proteinase with a cysteine residue in its active site. This enzyme promotes the growth, invasion, and metastasis of cancer cells by catalyzing the degradation of the interstitial matrix and basement membranes; this allows cancer cells to invade locally and to metastasize [24, 68]. Cathepsin B also plays an important role in a variety of pathologies, including inflammation, pancreatitis, osteoarthritis, tumor angiogenesis, apoptosis, and neuronal diseases [6, 15, 19, 29, 31, 33, 49, 74]. In addition, this enzyme markedly enhances infection by the Ebola virus by converting the 130-kDa viral glycoprotein GP1 to a 19-kDa species [65]. Because of the role of cathepsin B in disease development, including cancer cell proliferation and virus infection, studies of cathepsin B inhibitors from marine isolates of *Pseudomonas* should be intensified.

Diversity of marine *Pseudomonas*

Ecological diversity of marine *Pseudomonas*

Marine isolates of *Pseudomonas* are found in diverse ecosystems, including coastal regions, the deep sea, and more extreme environments. Marine *Pseudomonas* also can be found as bacterioplankton in seawater, in association with other marine organisms, and in sea sediment. The production of marine secondary metabolites can be viewed in an ecological context [25]. Thus, the diversity of *Pseudomonas* isolated from a wide range of marine ecosystems suggests that these organisms may produce novel and diverse bioactive substances.

P. fluorescence is usually found as bacterioplankton in seawater [22, 54], but Borges et al. [10] isolated the bacterium with denitrifying activity from a biofilter installed at a marine recirculation aquaculture system. Marine *Pseudomonas* bacterioplankton have great biodiversity as revealed by Hagström et al. [30] when they isolated several bacterial strains closely related to *P. slutzeri, P. putida, P. megulae, P. anguilliseptica, P. gessardi, P. azotoformans*, and *P. veronii* from the Baltic and Weddell Seas. Danovaro et al. [23] used automated ribosomal intergenic spacer analysis (ARISA) to determine bacterial diversity in the aquatic environment. Specifically, seven *Pseudomonas* spp. strains were isolated from five different environmental samples. Analysis of 16S rDNA indicated that isolate 1 was closely related to *P. veronii* and *P. marginalis*, with 98% sequence similarity. Isolates 2 and 3 were closely related to *P. aurantiaca* (97%). Isolate 5 was close to *P. chloritidismutans* (99%) and *P. stutzeri* (99%). Two other isolates, strains 6 and 7, were closely related to *P. aeruginosa* (99%). Notably, isolate 4 was not closely related to any specific species of *Pseudomonas*, but relatively close to *Pseudomonas* sp. strain MFY152 (98%).

In the marine ecosystem, *Pseudomonas* also associates with other micro- and macroorganisms. Alavi et al. [2] analyzed the bacterial community associated with *Pfiesteria*-like dinoflagellate cultures and identified two 16S rDNA clones closely related to *P. hibiscicola* and *P. oleovorans* with 99% sequence similarities. Thakur et al. [72] isolated two marine antibacterial-producing *Pseudomonas* sp. (strains PB1 and PB2) from a *Suberites domuncula* sponge primmorph; the *n*-butanol extract of these *Pseudomonas* strains has both antibacterial activity and autoinhibitory activity. Berg et al. [9] isolated *Pseudomonas* sp. 13BT from an axenic culture of *Aureococcus anophagefferens* (Pelagophyceae), a eukaryotic picoplankton. This isolate is closely related to *P. luteola* (formerly *Chryseomonas luteola*), with 99% 16S rDNA sequence similarity. This bacterium is able to hydrolyze urea and acetamide, but its capability is very limited for aminopeptides and citobiose. Anand et al. [4] screened for antibiotic-producing marine bacteria associated with sponges from the waters off the coast of southeast India and isolated a bacterium, strain SC11, which is closely related to *Pseudomonas* based on the 16S rDNA sequences.

Diverse *Pseudomonas* spp. are also found in association with corals. Brück et al. [14] isolated bacterial strains from Azooxanthellate deep-water octocorals *Leptogorgia minimata*, *Iciligorgia schrammi*, and *Swiftia exertia*, which grow at moderate depth (40–100 m). Based on 16S rDNA sequence analysis, the isolates are thought to be related to *P. citronellolis* (for strain LM1305), *P. oryzihabitans* (for strain LM1405), *P. putida* (for strain LM1505), *P. aeruginosa* (for strains LM1605, LM2705, DQ517271, and SE1005), *P. pachastrellae* (for strain LM2805), and *P. pseudoalcaligenes* (for strain LM2905). The other isolates, IS1005, LM1605, LM3005, LM3105, LM3205, and LM3305, are not related to any specific species of *Pseudomonas*, but can be included in this genus.

Marine *Pseudomonas* has been found to be a member of the microorganism community in marine extreme environments. Barotolerant marine *Pseudomonas* sp. BT1 was isolated in deep water (4,418 m) in a Japanese ocean trench [39]. This bacterium was closely related to *P. stutzeri*, based on the 98% sequence similarity of the 16S rDNA sequences. Zeng et al. [81] isolated a deep-sea

psychrophilic bacterium identified as *Pseudomonas* sp. DY-A that produces a cold-active serine alkaline protease. Radjasa et al. [56] failed to isolate any *Pseudomonas* strains from deep-sea (1,000-9,671 m) samples in the Northwestern Pacific Ocean. Although two bacterial strains that are closest to *P. beijirinckii* based on 16S rDNA sequence analysis were recovered from surface samples at the same location, these strains are distantly related to and likely distinct from the genus *Pseudomonas* due to the low 16S rDNA similarity (93.6%).

P. stutzeri has diverse ecological origins in both terrestrial and marine ecosystems. This phenotypically and genetically heterogeneous group is composed of several genomovars [8, 21, 28, 45]. Marine isolates of *P. stutzeri* have been reported in marine sediment in Barcelona, Spain [11–13, 60, 61], in sea sediment in Dangast, Germany [67], and in marine sediment at 11,000-m depth in the Mariana Trench [70]. Marine isolates of *P. stutzeri* have also been reported to live in the Black Sea (southwest) at 120-m depth [69], in a deep-sea hydrothermal vent in the Galapagos Rift [62] and in the Ariake Sea tideland, Japan [40]. Strains of this species can degrade naphthalene [11–13, 60, 61], oxidize thiosulfate [69], and sulfur [62], denitrify [40], and produce urea [73] and antibiotics [1, 75–77]. Amachi et al. [3] also have isolated *Pseudomonas* strain SCT, a dissimilatory iodate-reducing bacterium closely related to *P. stutzeri*. Two strains of marine *Pseudomonas* closely related to *P. stutzeri* have been isolated from the chemocline of a hypersaline deep-sea basin (Urania Basin, Mediterranean Sea) [64].

P. aeruginosa is a well-known pathogenic bacterium. It is generally regarded as a freshwater or terrestrial bacterium, as it is frequently isolated from these environments as well as from diseased organisms. However, Kimata et al. [44] showed that this *Pseudomonas* sp. also lives in a marine environment. The detection by two outer membrane lipoprotein genes specific to *P. aeruginosa*, *oprI* and *oprL,* using API 20 NE kit and 16S rDNA sequence analysis, revealed that *P. aeruginosa* inhabits Tokyo Bay. Subsequent work indicated that this species can be isolated from open ocean as well [42]. Jamil et al. [36] isolated a bacterium strain CMG607w from sediment of the Karachi coast, Pakistan. The strain that produces polyhydroxyalkanoate (PHA) is identified to be *P. aeruginosa* with 98% 16S rDNA sequence similarity.

In summary, marine *Pseudomonas* can be isolated from diverse ecological environments. The unique ecological characteristics of their environment may influence the production of secondary metabolites by the bacteria. However, studies of *Pseudomonas* isolated from diverse marine environments, as well as studies of the bioactive substances produced by these bacteria, are still rare.

Phylogenetic relationship of marine isolates of *Pseudomonas*

A search of 16S rDNA sequences in the GenBank, DDBJ, and EMBL databases shows that hundreds of marine isolates of *Pseudomonas* have been described. However, the number of reported marine *Pseudomonas* sp. is relatively small compared to terrestrial *Pseudomonas* sp. Within *Pseudomonas* (sensu stricto), the marine isolates include *P. stutzeri*, formerly *P. perfectomarina* [7, 61], *P. alcaligenes*, *P. pseudoalcaligenes* [53], *P. fluorescens* [51], *P. alcaliphila* [80], *P. aeruginosa* [44], *P. xanthomarina* [59], *P. pachastrellae* [58], and *P. marincola* [57].

P. alcaliphila, a facultative psychrophilic alkaliphile, was isolated from seawater [80]. *P. pachastrellae* was isolated from a deep-sea sponge specimen from the Philippine Sea at a water depth of 750 m [58]. Romanenko et al. [59] also isolated *P. xanthomarina* from ascidians specimens in the Sea of Japan and *P. marincola* from a deep-sea brittle star in the Fiji Sea [57].

In bacterial taxonomy, the use of 16S rDNA as a phylogenetic marker has been developed for determining intergeneric relationships because the gene evolves at an extremely slow rate. The 16S rDNA sequences of *Pseudomonas* were used by Anzai et al. [5] to evaluate the affiliation, group, and reclassification of *Pseudomonas* sp. We constructed the phylogenetic tree of marine *Pseudomonas* sp. based on 16S rDNA sequences retrieved from the GenBank, DDBJ, and EMBL databases, and it shows that marine isolates of *Pseudomonas* can be divided into two main clusters. The first cluster consists of *P. alcaliphila*, *P. alcaligenes*, *P. aeruginosa*, *P. xanthomarina*, and the group of *P. stutzeri*. The second cluster consists of *P. marincola, P. pachastrellae, P. fluorescens, P. fulva,* and *P. veronii* (Fig. 2). This result largely confirmed an earlier report that the phylogenetic tree based on the sequences of 16S rDNA of species in the genus *Pseudomonas* (sensu stricto) has two main clusters [5]. The phylogenetic tree presented here (Fig. 2) includes several recently reported novel marine *Pseudomonas* sp.: *P. alcaliphila* [80], *P. xanthomarina* [59], *P. pachastrellae* [58], and *P. marincola* [57].

Marine isolates of *Pseudomonas* that produce bioactive substances are likely to be dominated by members in the second cluster. However, the relationship between the ability to produce bioactive substances and position on the phylogenetic tree is still unclear, probably because so few marine *Pseudomonas* sp. have been studied extensively. In the second cluster, bacteria reported to produce bioactive substances include *Pseudomonas* sp. AMSN [34, 35, 38], *Pseudomonas* sp. MP-6 (GenBank/DDBJ/EMBL databases), *P. fulva* An10 (GenBank/DDBJ/EMBL databases), *P. fluorescens* PS7 (GenBank/DDBJ/EMBL databases),

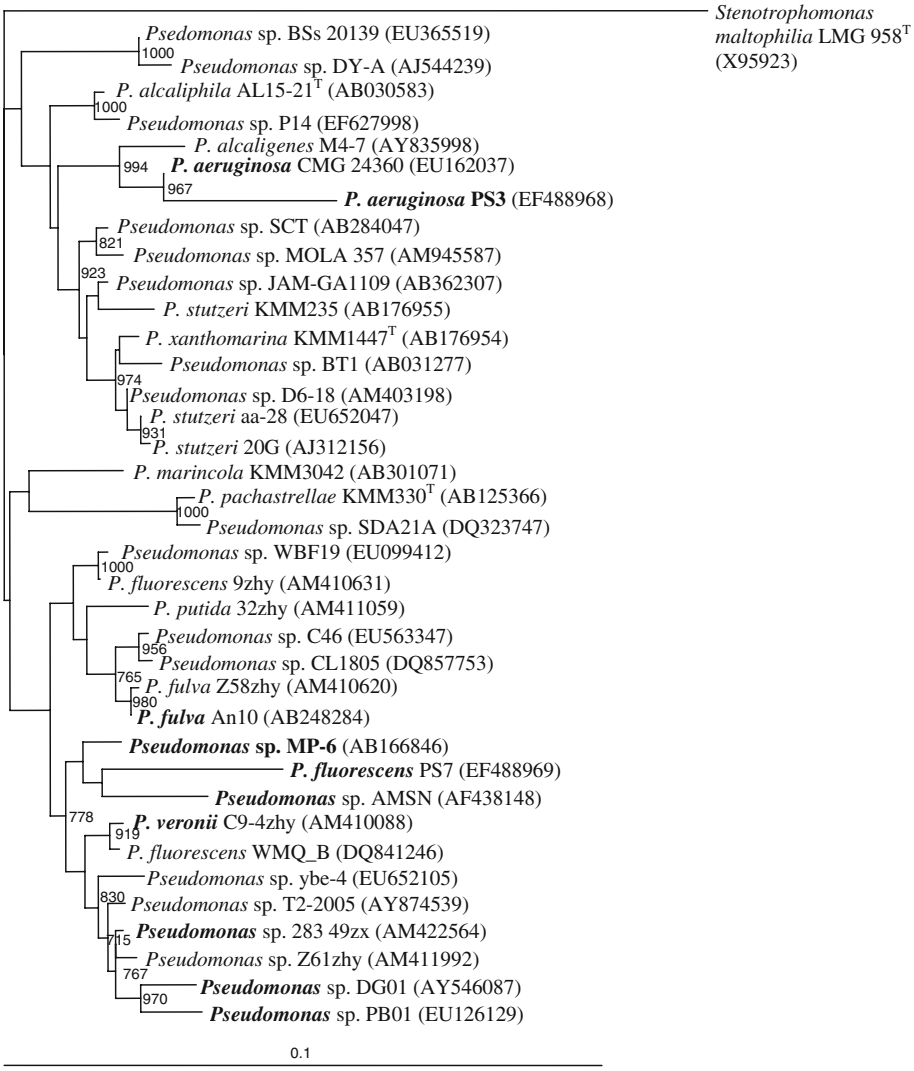


Fig. 2 Phylogenetic tree based on the 16S rDNA of marine isolates of *Pseudomonas*. The branching pattern was generated by the neighbor-joining method. Bootstrap values of 700 or more (from 1,000 replicates) are indicated at the nodes. Sequence accession numbers are in *parentheses*. *Bar* indicates 0.01 Knuc unit. Bacteria that produce bioactive substances are shown in *bold*

Pseudomonas sp. DG01 (GenBank/DDBJ/EMBL databases), *Pseudomonas* sp. PB01 [32], *P. veronii* C9-4zhy (GenBank/DDBJ/EMBL databases), and *Pseudomonas* sp. 283 49zx (GenBank/DDBJ/EMBL databases). Two strains of *Pseudomonas* in the first cluster, namely *P. aeruginosa* CMG 24360 (GenBank/DDBJ/EMBL databases) and *P. aeruginosa* PS3 (GenBank/DDBJ/EMBL databases), are known to produce bioactive substances.

Conclusions

There are few reports of bioactive substance-producing marine *Pseudomonas* sp. compared to those describing terrestrial species that produce bioactive metabolites. However, some bioactive substances with novel biological activities and mechanisms have been extracted from marine isolates of *Pseudomonas*, and some of these metabolites have antimicrobial properties. The genetic and ecological diversity of *Pseudomonas* suggest that marine isolates are a potential source of bioactive metabolites that could form the basis of new medical therapies.

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