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Genomic Attributes of Novel Symbiont *Pseudovibrio brasiliensis* sp. nov. Isolated From the Sponge *Arenosclera brasiliensis*

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Sponge holobionts are defined as the host animals and their associated microbiomes. Both host and microbiome produce extracellular products that facilitate interaction within the holobiont. For example, microbes may provide nutrition for the animal host and protection against pathogens. The genomic study of bacterial cultures may shed light on the properties of novel symbiotic bacteria isolated from marine holobionts. In this study, we performed a genome-based analysis of *Pseudovibrio brasiliensis* Ab134^T isolated from the sponge *Arenosclera brasiliensis*. This novel strain is phylogenetically related to *Pseudovibrio denitrificans*. *In silico* DNA-DNA hybridization and calculation of the average amino acid identity between the strain Ab134^T and *P. denitrificans* JCM 12308^T showed <70% similarity and <95% identity, respectively. This novel bacterial species possesses genomic features that hint at several possible roles in symbiosis (e.g., production of secondary metabolites, including bromotyrosine-derived alkaloids) in sponge and coral holobionts. We also detected gene clusters encoding type III, type IV, and type VI secretion systems and 26 toxin-like proteins, including probable paralogs. Our results demonstrate the genome versatility of *P. brasiliensis* Ab134^T and the potential to attach to host cells, which may play a role in its symbiotic lifestyle.

Keywords: *Pseudovibrio*, genomic taxonomy, corals, sponges, secondary metabolites, fistularin-3

INTRODUCTION

The genus *Pseudovibrio* belongs to the order Rhodobacterales of the class *Alphaproteobacteria*. It comprises six species: *Pseudovibrio denitrificans* (Shieh et al., 2004), *Pseudovibrio ascidiaceicola* (Fukunaga et al., 2006), *Pseudovibrio japonicus* (Hosoya and Yokota, 2007), *Pseudovibrio axinellae* (O'Halloran et al., 2013), *Pseudovibrio hongkongensis* (Xu et al., 2015), and *Pseudovibrio stylochi* (Zhang et al., 2016). *Pseudovibrio* species have been reported worldwide and are found mainly as members of bacterial communities associated with marine invertebrate

holobionts, including tunicates (Sertan-De Guzman et al., 2007), flatworms (Xu et al., 2015), corals (Essack, 2001; Bondarev et al., 2013), sea squirts (Fukunaga et al., 2006), algae (Vizcaino, 2011), and a wide variety of sponges (Breitbart et al., 2003; Muscholl-Silberhorn et al., 2008; O'Halloran et al., 2013; Appolinario et al., 2016). They are also found as free-living bacteria in seawater (Shieh et al., 2004; Hosoya and Yokota, 2007). *Pseudovibrio* species are heterotrophic, facultative anaerobic, marine bacteria capable of denitrifying and fermenting a range of substrates (Romano et al., 2016).

Pseudovibrio species were reported as dominant in the culturable bacterial fraction of various marine sponges (Webster and Hill, 2001; Muscholl-Silberhorn et al., 2008; Bauvais et al., 2015). A symbiotic relationship appears to exist between *Pseudovibrio* and sponges (Taylor et al., 2007); however, it remains unclear whether these bacteria are sponge mutualists/commensalists or pathogens/parasites. *Pseudovibrio* has been isolated only from healthy sponges and other holobionts, except for a single study reporting the association of *Pseudovibrio* with diseased (bleached) scleractinian corals (Moreira et al., 2014). The abundance of *Pseudovibrio* strains decreases drastically in diseased sponges (Sweet et al., 2015). Some strains of *Pseudovibrio* may protect their hosts by inhibiting the growth of the sponge pathogen *Bacillus* (Webster and Hill, 2001; Esteves et al., 2017). Some strains of *Pseudovibrio* produce biologically active secondary metabolites with antimicrobial activity (Sertan-De Guzman et al., 2007; Penesyan et al., 2011; Vizcaino, 2011; Nicacio et al., 2017). Genomic analysis of ten *Pseudovibrio* strains isolated from marine sponges collected on the west coast of Ireland revealed a diverse repertoire of genes involved in prokaryote-eukaryote interactions, including potential toxin-immunity systems and secretion systems (Romano et al., 2016). However, it remains unclear whether these findings apply to other sponge holobionts or other locations.

Rua et al. (2014) analyzed the diversity and antimicrobial potential of culturable heterotrophic bacteria associated with the endemic sponge *A. brasiliensis* and isolated *Pseudovibrio* sp. Ab134^T. Bromotyrosine-derived alkaloids were recently reported from cultures of this strain (Nicacio et al., 2017). Fistularin-3, one of such metabolites induces apoptosis (Mijares et al., 2013) and exerts antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv and low cytotoxicity against macrophages (De Oliveira et al., 2006).

In the present study, we compared the genomic attributes of *Pseudovibrio* sp. Ab134^T with those of related *Pseudovibrio* species to identify genes with biotechnological potential and involved in symbiosis. Our results provide further evidence of *Pseudovibrio* spp. as members of the stable microbiome of sponge hosts. Furthermore, their usefulness as source of bioactives is highlighted with genomic and experimental evidence, which is advantageous since not all bacterial producers of such chemicals can be easily isolated, cultivated, and yields metabolites in laboratory conditions. We then classified this novel strain Ab134^T using a genome-based taxonomic analysis (Thompson et al., 2015), improving the present delineation of this group encompassing metabolically active members yet not identified to species level.

MATERIALS AND METHODS

Isolation of *Pseudovibrio* Strain

The *Pseudovibrio* strain was isolated as previously described (Rua et al., 2014). *Pseudovibrio* sp. Ab134^T has been deposited in the Collection of Environmental and Health (CBAS) at the Oswaldo Cruz Institute (IOC), FIOCRUZ (Rio de Janeiro, Brazil) (http://cbas.fiocruz.br/) and assigned the accession number CBAS 623^T. The strain was also deposited in the Collection of Aquatic Microorganisms (CAIM) in Mazátlan, Sinaloa, Mexico (http://www.ciad.mx/caim/CAIM.html) and assigned the accession number CAIM 1924^T.

Genome Sequencing, Assembly, and Annotation

Genomic DNA was extracted using a previously described method (Pitcher et al., 1989). DNA libraries were built using the Nextera DNA Sample Preparation Kit (Illumina, San Diego, CA, USA). The DNA was purified using AMPure XP beads and quantified using the fluorometric Qubit dsDNA HS Assay Kit (Life Technologies). Quantification of libraries was performed with the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and the KAPA Library Quantification Kit (Kapa Biosystems, Wilmington, MA, USA). Library size distribution was determined using the Agilent 2100 Bioanalyzer. Genome sequencing was performed using the Illumina MiSeq platform (paired-end sequencing, 2 × 300 base pairs). The sequences obtained were preprocessed using PrinSeq software to remove small reads (<35 bp) and low-score sequences (Phred score <30) (Schmieder and Edwards, 2011). Two programs assembled high-quality reads: A5-miseq (Coil et al., 2015) assembled the sequence data, and then the generated contigs and the singletons were used as input for the CAP3 sequence assembly program (Huang and Madan, 1999). Functional annotation was carried out by the Rapid Annotations using Subsystem Technology (RAST) platform (Aziz et al., 2008).

Phylogenetic Analysis Based on 16S rRNA and *recA* Genes

The 16S rRNA gene of strain Ab134^T was obtained as previously described (Moreira et al., 2014; Rua et al., 2014) and compared to known sequences in the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST). The closest matches were included in the phylogenetic analysis. The 16S rRNA gene identities were calculated using Jalview V.2 (Waterhouse et al., 2009). Pairwise and multiple alignments were performed using ClustalW (Larkin et al., 2007). Evolutionary history was inferred using the neighbor-joining method (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 1,000 replicates was taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000), and evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

The *recA* gene sequences for *Pseudovibrio* genomes available on GenBank (*P. denitrificans* JCM 12308^T, *P.* sp. FO-BEG1, *P. axinellae* Ad2^T, and *P. hongkongensis* UST20140214-015B^T) were analyzed using the BLASTN algorithm (Altschul et al., 1990). Multiple alignment, phylogenetic reconstruction, and bootstrap consensus were conducted as described above for the 16S rRNA genes (Felsenstein, 1985; Saitou and Nei, 1987; Larkin et al., 2007; Tamura et al., 2013). Accession numbers are listed in **Table S1**.

Microbial Genomic Taxonomy

In silico DNA-DNA hybridization analysis was carried out by genome-to-genome comparison (Auch et al., 2010). Average nucleotide identity (ANI) was calculated as previously described (Thompson et al., 2013). The intraspecies genomic relatedness ranged from 95 to 100% ANI. Genome distance was calculated using a genome-to-genome distance (GGD) calculator (Meier-kolthoff et al., 2016), with intraspecies genomic similarity ranging from 70 to 100%.

Genome-Based Phenotype

Phenotype analysis was based on genome sequences (Amaral et al., 2014), focusing on eight biochemical characteristics that have been used to identify *Pseudovibrio* species (Shieh et al., 2004; Hosoya and Yokota, 2007). For each characteristic, we searched for the corresponding genes. If one or more genes involved in a phenotype was present in the genome, the organism was considered positive for this phenotype. Genes encoding proteins involved in those characteristics were detected by using the RAST program (Overbeek et al., 2014). Genes associated with related biochemical pathways were identified with the BLASTP algorithm (Altschul et al., 1990). We used the antiSMASH 2.0 software pipeline (Weber et al., 2015) to identify secondary metabolite biosynthesis clusters in the whole genome sequences of *P. brasiliensis* Ab134^T and *P. denitrificans* JCM 12308^T. The metabolic features of *P. denitrificans* JCM 12308^T and the Ab134^T strain were compared using the RAST platform (Overbeek et al., 2014).

Search for Homologous Genes Related to Symbiosis

In order to identify genes related to symbiosis in *P. brasiliensis* Ab134^T, secretion system types III, IV, and VI and toxin-like proteins were predicted using two different approaches. The search for secretion systems was carried out using T346Hunter, a web-based tool for the prediction of type III, type IV, and type VI secretion systems (Martínez-García et al., 2015). Detection of toxin-like proteins was carried out using BLASTP and the Virulence Factors Database (VFDB; Chen et al., 2016). For the BLASTP analysis, amino acid sequences from *P. brasiliensis* Ab134^T were predicted using Prodigal software v2.6.2 (Hyatt et al., 2010) with default parameters and used as query sequences. Results annotated as "toxin" were used in the subsequent analysis. Genes associated with the secretion systems of *P. brasiliensis* Ab134^T and *P. denitrificans* JCM 12308^T were detected out using the same approach.

In Vitro Phenotypic Characterization

Growth of *Pseudovibrio* sp. Ab134^T at different NaCl concentrations (0% and 5.0% [w/v]), temperatures (4, 10, and 13–44°C), and pH values (5–10, adjusted with NaOH or HCl) were tested on marine agar medium. Plates were incubated at 27°C (or the test temperature) for up to 7 days in triplicate. Motility was evaluated on semisolid marine agar and stab inoculation into tubes and confirmed by phase contrast microscopy, which was also used to evaluate Gram staining, shape, and diameter. Features that differed between Ab134^T and the reference strains of the three *Pseudovibrio* species are listed in **Table S2**.

Antimicrobial Activity

Strains and Growth Conditions

The following three indicator strains were selected for the antimicrobial production assay: *Bacillus subtilis* CECT 461^T (Rua et al., 2014), the coral pathogen *Vibrio coralliilyticus* P1, and the human pathogen *Vibrio parahaemolyticus*. *V. coralliilyticus* P1 produces proteases, including a zinc-containing metalloprotease (Santos Ede et al., 2011). *V. parahaemolyticus* was PCR-positive for the presence of hemolysin genes *tlh* and *trh* (data not shown). The indicator strains were activated in marine/LB agar (1:0.5) at 30°C overnight, and the test strain, *P. brasiliensis* Ab134^T, was activated in marine agar at 30°C overnight.

Antimicrobial Production Assay

P. brasiliensis Ab134^T was spotted onto marine agar; the spots were ∼1 cm in diameter. After 72 h, the cells were killed by exposure to chloroform vapor for 1–2 h, as follows. A piece of cotton was saturated with 1 mL chloroform and placed on the plate lid. The plate containing agar and cells was then inverted, placed on the lid, and incubated for 1–2 h. Indicator strains were inoculated in marine/LB soft agar, which was poured onto the plates with the dead cells (test strain). After incubation at 30°C for 24 h, clear zones around the spots of dead cells indicated that the test strain had produced an antimicrobial compound. This assay was performed in triplicate.

RESULTS

General Characteristics and Metabolic Features Predicted in the *P. brasiliensis* Ab134^T Genome

A total of 1,967,588 paired-ends reads for the Ab134^T strain were generated. The genome assembly resulted in 39 contigs, and the coverage was 164-fold. Estimated genome size was 5,975,631 bp, and the number of coding sequences estimated by Prodigal was 5476. Of the 66 RNA sequences, 61 were tRNAs, and 5 were rRNAs. Functional annotation revealed that most of the 2,260 genes identified were assigned to carbohydrates (301), amino acid and derivatives (281), and cofactors, vitamins, prosthetic groups, and pigments (234) (**Figure S1**).

Our results showed that *P. brasiliensis* Ab134^T is phylogenetically and genetically related to *P. denitrificans* (**Figure S2** and **Table S3**). A functional comparison between the *P. denitrificans* JCM 12308^T and *P. brasiliensis* Ab134^T genomes

revealed 51 genes unique to *P. denitrificans* JCM 12308^T, including those related to urea decomposition, acetyl-CoA conversion to butyrate, menaquinone and phylloquinone biosynthesis, curli production, dihydroxyacetone kinases, and the G3E family of P-loop GTPases (metallocenter biosynthesis, urease accessory). The 34 genes unique to the *P. brasiliensis* Ab134^T genome included those related to phages and prophages, maltose and maltodextrin utilization, transport of nickel and cobalt, toxin-antitoxin replicon stabilization systems, and thioredoxin-disulfide reductase (**Table S4**). Genes shared by *P. brasiliensis* Ab134^{T}and *P. denitrificans* JCM 12308^T included those related to nitrogen metabolism (dissimilatory nitrite reductase, nitrate and nitrite ammonification) (**Table S5**) and fermentation processes (butanol biosynthesis, mixed acid, lactate, acetolactate synthase subunits, and acetyl-CoA fermentation to butyrate) (**Table S6**), suggesting the potential of *P. brasiliensis* Ab134^T to carry out denitrification and fermentation in the sponge holobiont.

Secondary Metabolite Biosynthesis Clusters Analysis

Using the antiSMASH program we identified clusters related to secondary metabolite biosynthesis (**Figures 1**,**2** and **Figures S3**,**S4**) and found differences in gene cluster abundance and diversity between *P. denitrificans* JCM 12308^T and *P. brasiliensis* Ab134^T. The nine clusters identified in *P. denitrificans* JCM 12308^T are involved in the biosynthesis of bacteriocins (2), terpene (1), non-ribosomal peptide synthases (NRPSs) (2), hybrid NRPS-polyketide synthase (PKS)-T1 (1), hybrid PKS T1-bacteriocin (1), homoserine-lactone (1), and T3PKS-T1PKS (1). The five clusters identified in *P. brasiliensis* Ab134^T were assigned to the biosynthesis of terpene (1), bacteriocins (2), PKS (1), and NRPS (1). Some gene clusters showed no similarity to known *Pseudovibrio* gene clusters.

Terpene Gene Cluster

Cluster 1 (20.9 kb) contained 20 genes predicted to be involved in terpene production. This cluster showed a 54% sequence similarity to genes in *Pseudovibrio* sp. FO-BEG1 and 51% sequence similarity to genes in *Pseudovibrio* sp. JE062 (**Figure S3**). Both strains (FO-BEG1 and JE062) were genetically characterized as *P. denitrificans* (Bondarev et al., 2013; Romano et al., 2016).

Bacteriocin Gene Cluster

Clusters 2 and 3 were related to bacteriocin production. Cluster 2 (19.3 kb) shared only 10% sequence similarity to FO-BEG1 genes, and cluster 3 (11 kb) shared 24% sequence similarity to JE062 and FO-BEG1 genes suggesting that they represent two new gene clusters (**Figure 1**).

Polyketide Synthase and Non-ribosomal Peptide Synthetase Gene Clusters

In the *P. brasiliensis* Ab134^T genome we identified a 7.2-kb cluster containing 41 genes predicted to be involved in NRPS production. This cluster shared only 18% sequence similarity to genes in *Pseudomonas* sp. GM25 PM124 (**Figure S4**). The best characterized cluster detected was a hybrid PKS3-PKS1 (**Figure 2**). Cluster 5 (63.5 kb) contained 120 genes predicted to be involved in the production of this hybrid PKS3-PKS1, and shared 91 and 89% sequence similarity to genes in JE062 and FO-BEG1, respectively (**Figure 2**). The presence of PKS and NRPS genes is often associated with the production of bioactive secondary metabolites.

Genome analysis of strain Ab134^T revealed novel features that allow it to thrive in the sponge holobiont. Using HMMER3 (Eddy, 2009) we identified four types of enzymes that may be related to bromotyrosines' biosynthesis, including bromoperoxidases, S-adenosyl-L-methionine-dependent methyltransferases and ATP-grasp ligases such as glutathione synthetase and friulimicin.

Secretion Systems and Prokaryote-Eukaryote Interaction

We identified four gene clusters encoding non-flagellar type III secretion systems (T3SSs) in the *P. brasiliensis* Ab134^T genome (**Figure S5**). All essential genes were present in three of these clusters. Genes in the four clusters showed high similarity to those from *P. denitrificans* JCM12308^T (**Figure S5**). The main difference was the presence of three genes from other secretion systems. T3SS cluster 1 (T3SS-1) contained the gene *tfc4* (155 amino acids), which is characteristic of T4SS. T3SS cluster 3 (T3SS-3) and cluster 4 (T3SS-4) contained *vasH* genes (854 and 421 amino acids, respectively), which are characteristic of T6SS. We also identified one cluster that appears to encode 12 proteins of a T4SS, containing mainly *virB* genes and *trb* (**Figure S6**).

We identified two clusters that encode T6SS genes, both including *vgrG* and *hcp* genes (**Figure S7A**). The distribution of effector protein-coding genes was similar to that of the two T6SS clusters identified in the *P. denitrificans* JCM 12304^T genome (**Figure S7B**), but the number and arrangement of genes differed. Both Ab134^T and *P. denitrificans* JCM 12304^T genomes contained 16 core component genes in the first cluster (T6SS-1). Whereas in the second cluster (T6SS-2), Ab134^T and *P. denitrificans* JCM 12304^T genomes showed five and 19 core component genes, respectively (**Figures S7A,B**). Taken together, these results suggest specific interactions with eukaryotes and the potential ability to target host cell machinery.

Potential Toxin-Like Protein-Coding Genes in *P. brasiliensis* Ab134^T Genome

A wide range of potential toxin-like protein-coding genes was identified in the *P. brasiliensis* Ab134^T genome. Of the 25 hits retrieved from a BLASTP search against the VFDB, 15 genes were unique. The genes *argK*, *frpC*, *rtxA,* and *syrE* were present in multiple copies, suggesting the presence of paralogs (**Table S7**).

Phylogenetic and Genomic Delineation of *Pseudovibrio brasiliensis* sp. nov.

Ab134^T clustered tightly with other *Pseudovibrio* spp. based on the 16S rRNA gene sequences analysis (**Figure S2A**), showing

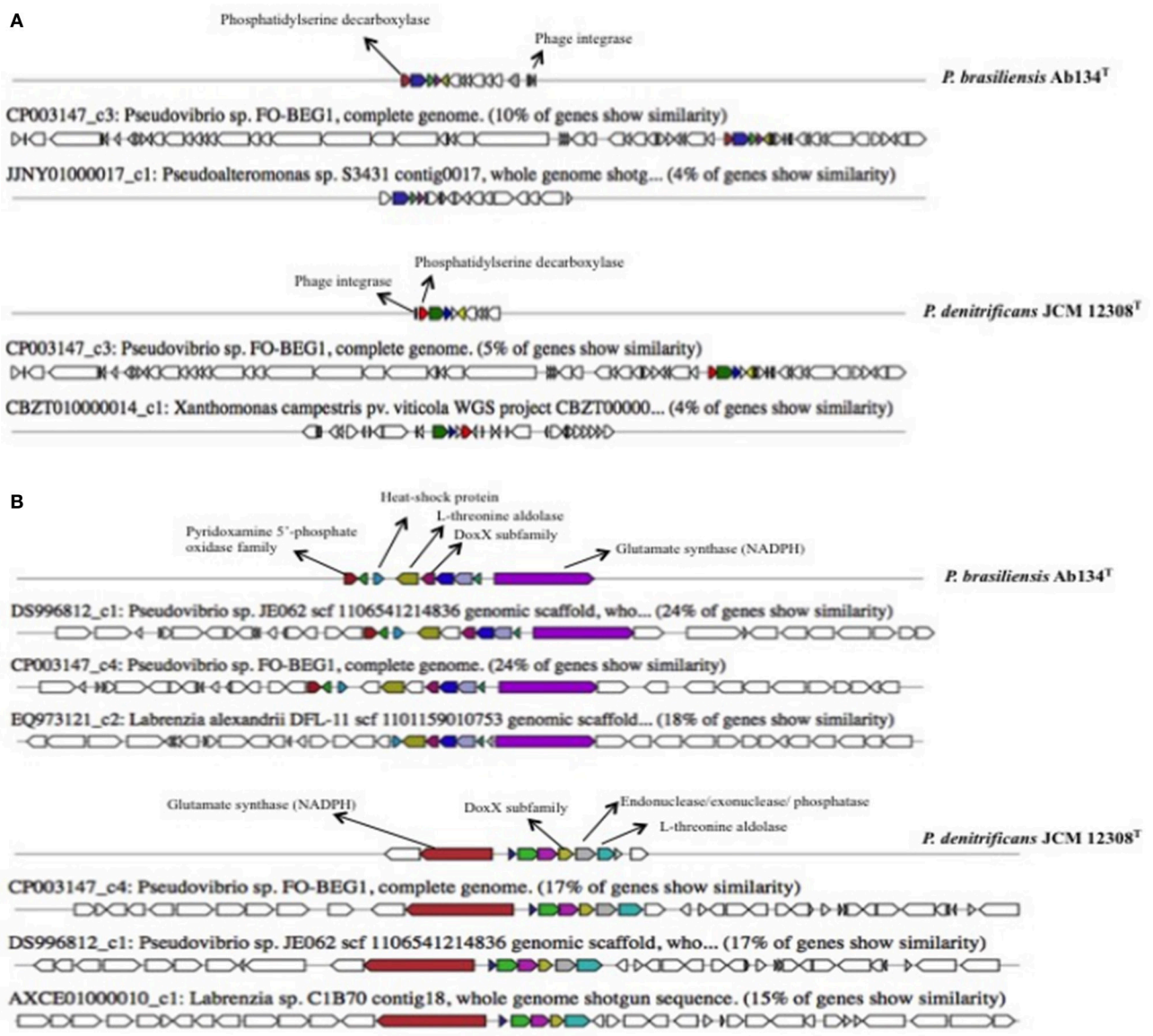

FIGURE 1 | Bacteriocin biosynthesis pathway (cluster 2) **(A)** and novel bacteriocin pathway (cluster 3) **(B)** identified in the *P. brasiliensis* Ab134^T genome.

identity values to *P. denitrificans* strains of 99.4% (JE062, NW001) and 99.5% (**JCM12308**^{T}, FO-BEG1, MBIC3368). Strains JE062 and FO-BEG1 shared almost identical 16S rDNA gene sequences (99.9%) with *P. denitrificans* DN34^T. Based on the phylogenetic analysis of *recA*, Ab134^T shared only 93% sequence identity with *P. denitrificans* JCM 12308^T and FO-BEG1 (**Figure S2B**), suggesting that Ab134^T represents a new species of the genus *Pseudovibrio*. A formal description is provided in the Supplementary Material.

Pairwise genomic comparisons between *P. brasiliensis* Ab134^T and *P. denitrificans* JCM 12038^T (Shieh et al., 2004) showed that they share only 93% ANI and 54.3% (±3) GGD similarity (*in silico* DNA-DNA hybridization) (**Table S3**). A bacterial species is defined as a group of strains that share ≥98.7% 16S rRNA gene sequence similarity, >95% ANI and >70% GGD similarity (Thompson et al., 2015). Based on phylogenetic analysis, *in silico* DNA-DNA hybridization, ANI, and differential phenotypic characteristics, strain Ab134^T is proposed as the type strain of a novel species, for which the name *Pseudovibrio brasiliensis* sp. nov. is proposed.

DISCUSSION

We report the genomic characterization of *P. brasiliensis* Ab134^T previously isolated from the marine sponge *A. brasiliensis* (Rua et al., 2014). The genome size of 5.9 Mb, with a G + C content of 52.1%, is consistent with previously reported values for this genus of ∼3.6–6.2 Mb (Romano et al., 2016; Zhang

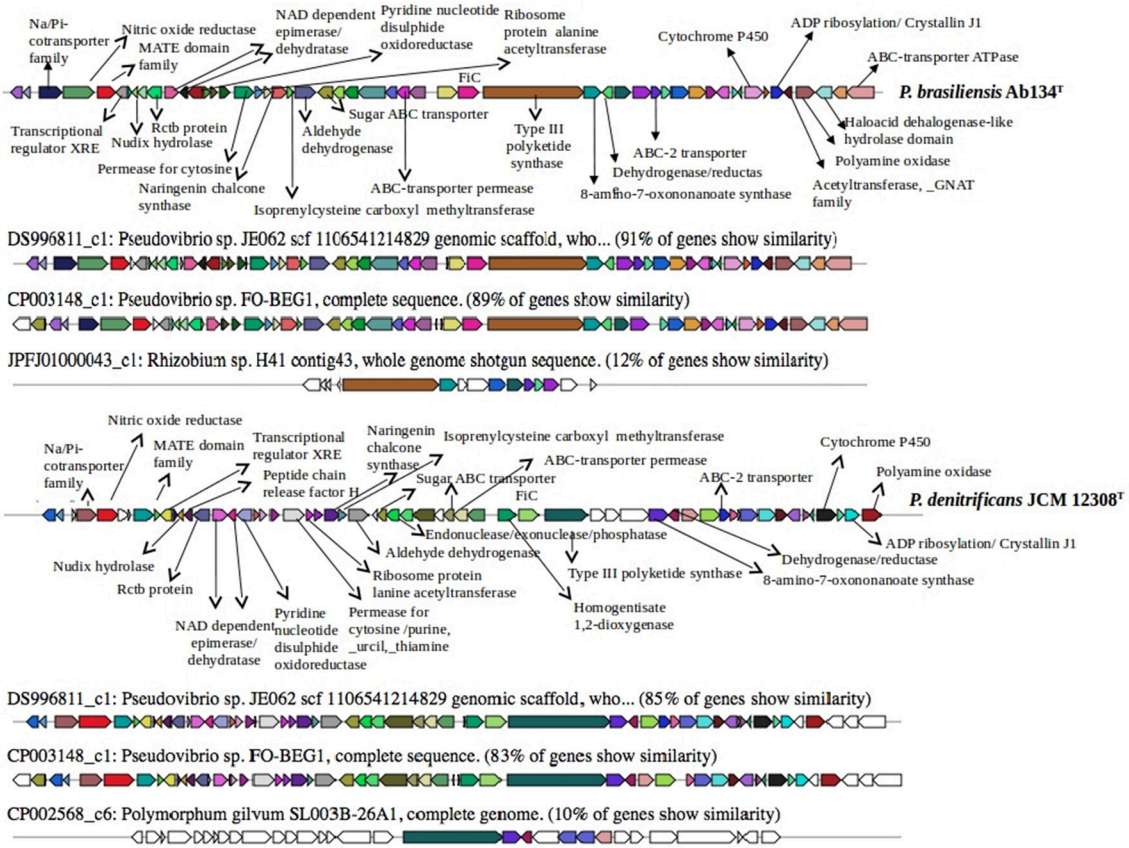

FIGURE 2 | PKS3-PKS1 pathway cluster identified in the *P. brasiliensis* Ab134^T genome and in the *P. denitrificans* JCM 12308^T genome.

et al., 2016). The secretion systems detected in *P. brasiliensis* genome represent a vast repertoire to facilitate interaction with its hosts. They are similar to those of the strains of *P. denitricans* FO-BEG1 and JE062 (Romano et al., 2016). However, *P. brasiliensis* possesses a distinct phylogenetic position and unique secondary metabolite and toxin-like genes (e.g., bromotyrosine-related genes). These genes may be useful for host-microbe interactions within the sponge, tunicate, and coral holobionts (Alex and Antunes, 2015).

Secondary Metabolites May Be Important For Holobiont Homeostasis

Secondary metabolites and toxins may complement each other to promote holobiont homeostasis. Secondary metabolites (e.g., bacteriocins, terpenes, and NRPS) are encoded by gene clusters, whereas toxins are encoded by a few genes or a single gene (e.g., *argK, frpC, rtxA*) (Sertan-De Guzman et al., 2007; Penesyan et al., 2011; Vizcaino, 2011; O'Halloran et al., 2013).

Bacteriocins are ribosomally synthesized antimicrobial peptides that are lethal to closely related bacteria. Bacteriocin producers are protected from the effects of these peptides by a specific immunity protein(s) (Cotter et al., 2005). Bacteriocins have been used extensively as preservatives in the food industry (Deegan et al., 2006) and have been identified as potential alternatives to antibiotics (Piper et al., 2009). Bacteriocins may also serve as anti-competitor toxins, enabling a strain or species to invade an established microbiome (Riley and Gordon, 1999; Lenski and Riley, 2002; Riley and Wertz, 2002). In sponge holobionts, bacteriocins protect the host against pathogenic bacteria, and bacteriocin-producing bacteria may prevent the dissemination of pathogens by occupying the same ecological niche (Desriac et al., 2010).

Kennedy et al. (2008) reported that *Pseudovibrio* cultures from the marine sponge *Haliclona simulans* contain both putative PKS and NRPS genes, suggesting a potential for secondary metabolite production. These strains exhibited antimicrobial activity against methicillin-resistant *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *B. subtilis*. *P. brasiliensis* Ab134^T harbored PKS and NRPS gene cluster and exhibited antimicrobial activity against *B. subtilis* (Rua et al., 2014) and the pathogens *V. coralliilyticus* P1 and *V. parahaemolyticus* (this study).

P. brasiliensis Ab134^T also produces bromotyrosine-derived alkaloids (Nicacio et al., 2017). Additional enzymes involved in secondary metabolism were detected in this study. Experiments aiming to elucidate the biosynthetic gene clusters responsible

for these alkaloids are in progress and will be reported in due time.

Putative Roles Played by Toxins

Toxin genes detected in multiple copies include *argK, frpC, rtxA,* and *syrE*. *argK* encodes an ornithine carbamoyltransferase that confers self-resistance to phaseolotoxin, responsible for halo blight in beans (*Phaseolus vulgaris* L.), caused by *Pseudomonas syringae* pv. phaseolicola, (Mosqueda et al., 1990). This gene is known to be horizontally transferred (Sawada et al., 2002) and the product ArgK protects phaseolotoxin producers (self-resistance) in part by providing an alternative Arginine source (Chen et al., 2015). L-arginine is produced by bacterial fermentation and the main applications are in flavor and pharmaceuticals. Arginine overproducing strains has been a research target for the past decades (Lu, 2006). Another gene that was first described as a virulence determinant of *P. syringae* pv. syringae B301D was present in two copies. *syrE* is a phytotoxin gene present within the syringomycin cluster. Syringomycin is a cyclic lipodepsinonapeptide (Guenzi et al., 1998). This protein forms pores in plasma membranes, leading to electrolyte leakage (Bender et al., 1999) and might play a role in protecting the host (**Table S7**).

Multiple copies of *frpC* and *rtxA* were also detected. *frpC* encodes the iron-repressible repeat-in-toxin (RTX) protein FrpC, and *rtxA* encodes the structural toxin protein RtxA. Both belong to the RTX family, which consists primarily of cytotoxic pore-forming proteins (Schaller et al., 1999; Linhartová et al., 2010) acting as virulence determinants in many gram-negative pathogens. RTX proteins can also play a role in host protection as bacteriocins or by forming protective bacterial surface layers (S-layers) (Linhartová et al., 2010). RTX proteins exhibit additional biological activities as metalloproteases, lipases, pore-forming toxins, iron-regulated proteins, nodulation-related proteins and are involved both in bacterial adherence/motility and host-receptor interactions (Welch, 2001; de Souza Santos et al., 2015). RTX proteins are secreted by a T1SS via Sec-independent pathway used by gram-negative bacteria to transport proteins from the cytoplasm to the extracellular medium in a single step (Chenal et al., 2015). The presence of multiple copies of *frpC* and *rtxA* suggests a possible environmental role, including protection of the host and defense against other microorganisms and pathogens. The extracellular matrix of sponges is rich in proteoglycans, lamnin-like subunits, fibronectin, and other structural proteins (Harel and Tanzer, 1993; Özbek et al., 2010); thus, the multiple copies of RTX proteins may help to penetrate the sponge mesohyl.

Host-Microbe Interactions Mediated by Secretion Systems

A symbiotic relationship suggested between *Pseudovibrio* and marine invertebrates (Taylor et al., 2007) may involve interactions with holobiont host cells via secretion systems T3SS, T4SS, and T6SS. Secretion systems were first associated with pathogenic strains but have since been widely detected in symbiotic and free-living bacteria (Dale and Moran, 2006; Persson et al., 2009). Bacteria commonly use these three secretion systems to inject effector proteins in target cells, which facilitate colonization (Costa et al., 2015). For example, the non-flagellar T3SS (injectisome) enables gram-negative bacteria to deliver effector proteins into the cytoplasm of eukaryote hosts (Büttner, 2012). T4SS (VirB system), which was first identified in *Agrobacterium tumefaciens*, delivers toxins into host cells, as well as DNA that integrates into the host genome (Wallden et al., 2010), contributing to the genome plasticity and virulence of the bacteria (Voth et al., 2012).

Two genes (*virB* and *trb*) in the T4SS of *P. brasiliensis* Ab134^T are related to conjugation, suggesting a role in genome plasticity and environmental adaptation. These genes have been identified in other *Pseudovibrio* strains (Romano et al., 2016). Compared to *P. denitrificans* JCM 12308^T, the number of genes in the T4SS cluster was the same, but the gene annotation and rearrangement differed (**Figure S6**).

T6SSs are thought to help bacteria conquer an ecological niche (Ma et al., 2014; Russell et al., 2014; Kapitein and Mogk, 2017), and niche-specific distribution of T6SS effectors has recently been described (Egan et al., 2015; Romano et al., 2016). T6SSs appear to be involved in biofilm formation (Aschtgen et al., 2008), quorum sensing (Weber et al., 2009), interbacterial interactions (Hood et al., 2017), and anti-pathogenesis (Chow and Mazmanian, 2010; Jani and Cotter, 2017). The T6SS gene *impI* was detected only in the *P. brasiliensis* Ab134^T genome, which contained all core components of the T6SS, as described by Boyer et al. (2009).

CONCLUSION

P. brasiliensis Ab134^T displays bioactive secondary metabolite genes which might encode the antimicrobial(s) and bioactives already detected experimentally (this study; Rua et al., 2014; Nicacio et al., 2017). These features might prevent host colonization by pathogens and opportunistic organisms. The metabolic versatility of the species is demonstrated by several transporter systems characterized in its genome. Characterization of the genomic repertoire of *P. brasiliensis* shed light over putative mechanisms of host-microbe and microbe-microbes interactions within the sponge holobiont.

DATA DEPOSITION

This whole-genome shotgun sequencing project has been deposited at DDBJ/ENA/GenBank under the accession number MIEL00000000. The version described in this paper is version MIEL01000000.

AUTHOR CONTRIBUTIONS

FT and CT: designed and planned the study; AF and TF: performed the bioinformatics analyses; AM: performed 16S rRNA phylogeny and designed the antimicrobial production

assay; CS: performed tests. All authors contributed to the acquisition, analysis, and interpretation of the data. All authors contributed to the writing of the manuscript. All authors approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00081/full#supplementary-material

Figure S1 | General overview of the functional annotation of the *P. brasiliensis* Ab134^T genome.

Figure S2 | Neighbor-joining tree showing the phylogenetic position of *P. brasiliensis* Ab134^T based on 16S rRNA gene sequences **(A)** and *recA* gene sequences **(B)**. The bootstrap consensus tree inferred from 1,000 replicates is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches (>50%). Bar represents 0.005 nucleotide substitution rate (Knuc-values).

Figure S3 | Terpene metabolism gene cluster identified in the *P. brasiliensis* Ab134^T genome.

Figure S4 | NRPS pathway cluster identified in the *P. brasiliensis* Ab134^T genome.

Figure S5 | Representative genetic organization of type III secretion system (T3SS) in the *P. brasiliensis* Ab134^T genome **(A)** and *P. denitrificans* JCM 12308^T genome **(B)**.

Figure S6 | Representative genetic organization of type IV secretion system (T4SS) of *P. brasiliensis* Ab134^T genome.

Figure S7 | Representative gene context of type VI secretion system (T6SS) of the *P. brasiliensis* Ab134^T genome **(A)** and *P. denitrificans* JCM 12308^T genome **(B)**.

Table S1 | Genome and 16S rRNA accession numbers of *Pseudovibrio*, *Stappia stellulata* DSM 5886, *Pannonibacter indicus* HT23, *Labrenzia alexandrii* DFL-11.

Table S2 | Phenotypic characterization of *Pseudovibrio* species. 1—*P. brasiliensis* Ab134^T; 2—*P. denitrificans* JCM 12308 ^{T}; 3—*P. japonicus* NCIMB 14279^T; 4—*P. axinellae* Ad2^T; +, positive; −, negative.

Table S3 | Genomic characterization of *P. brasiliensis* Ab134^T. Identity (%) of the 16S rRNA (1) and *recA* gene sequences (2), average amino acid identity (AAI) (3) similarity (%) of the whole genome, and (4) *in silico* DNA-DNA hybridization (GGD) (4) between *Pseudovibrio* species.

Table S4 | Genes unique to *P. brasiliensis* Ab134^T, as assessed by functional comparison with *P. denitrificans* JCM 12308 ^{T} performed by the RAST server.

Table S5 | Genes related to nitrogen metabolism in the *P. brasiliensis* Ab134^T genome annotated by the RAST server.

Table S6 | Genes related to fermentation present in the *P. brasiliensis* Ab134^T genome based on functional annotation by the RAST server.

Table S7 | Probable toxin-like homologous of *P. brasiliensis* Ab134^T were identified by BLASTP search against the virulence factors database. Rows showing redundant functions are highlighted in bold.

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