Vibrio Gaelis sp. nov. Isolated From the Skin of Southern Atlantic Sharpnose-puffer (Canthigaster Figueiredoi)

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Short Report

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Abstract

Description of a Gram-negative, motile, circular-shaped bacterial strain, designated A621^T obtained from the skin of the pufferfish *Canthigaster figuereidoi* (Tetraodontidae Family), collected in Arraial do Cabo, Brazil. Optimum growth occurs at 20 - 28 °C in the presence of 3% NaCl. The Genome sequence of the novel isolate consisted of 4.224 Mb, 4,431 coding genes and G+C content of 44.5%. Genomic taxonomy analysis based on Average Amino Acid (AAI), Genome-to-Genome-Distance (GGDH) and phylogenetic reconstruction placed A621^T (= CBAS 741^T) into a new species of the genus *Vibrio* (*Vibrio gaelis* sp. nov.). The genome of the novel species contains 4 gene clusters (~56.17 Kbp in total) coding for different types of bioactive compounds that hint to several possible ecological roles in the pufferfish host.

Introduction

The family *Vibrionaceae* comprises 203 species (148 *Vibrio*, 33 *Photobacterium*, 6 *Aliivibrio*, 5 *Enterovibrio*, 4 *Salinivibrio*, 3 *Grimontia, 2 Thaumasiovibrio, 1* Paraphotobacterium and 1 *Echinimonas*) (Thompson & Gomez-Gil 2018). These bacteria are Gram-negative, fermentative, halophilic, mesophilic, chemoorganotrophic, and ubiquitous in the marine environment (Thompson et al. 2004). The bacterial genome sequence is used as a type material in current prokaryotic taxonomy (Thompson & Gomez-Gil 2018; Whitman et al. 2019). In this context, it is possible to describe new species and *in silico* phenotypes on the basis of the genome sequences. In addition, some vibrios originated from marine organisms have been deemed a relevant source of novel bioactive compounds (Machado et al. 2015). Therefore, these organisms are a rich source for biodiscovery.

The mucosal surface of the skin and its associated microbiota are an important primary barrier and offer defense against possible pathogens (Naik et al. 2012). Many strains of vibrios have been reported to cause skin diseases in fish, including those of the *V. ponticus* group (Soto-Rodriguez et al 2019; Ma et al 2017; Ongagna-Yhombi & Boyd 2013). However, due to its ubiquity and diversity, we can find new non-lethal or pathogenic strains, as phylogenetic neighbors of lethal strains and groups (Ina-Salwany et al 2018). As with many other fish, understanding the microbial communities of the skin and its dynamics can therefore imply in underlying changes in the health and fitness of pufferfishes (Llewellyn et al. 2018; Legrand et al. 2018; Legrand et al. 2019).

Our aim was to gain further insights on the vibrio microbiome of the marine pufferfish. We first reported the genome sequence of *Vibrio gaelis* A621^T. This vibrio was isolated from the skin of healthy pufferfish *Canthigaster figuereidoi* collected in Arraial do Cabo, Brazil (22°99'16"S; 41°99'76"W) in 2016. *C. figuereidoi* (common name: Southern Atlantic sharp nose-puffer) is endemic of Western Atlantic. It is distributed from the southern Caribbean to Santa Catarina, Brazil, including the oceanic islands of Atol das Rocas and Fernando de Noronha (Moura & Castro, 2002; Froese & Pauly, 2021). Its biotechnological importance has already been reported (Tonon et al. 2020). We then performed a genomic taxonomic investigation on this genome sequence. Additionally, we investigated subsystems for symbiosis-associated genes and related to the adaptative fitness (Bondarev et al. 2013). Finally, we demonstrated that this genome contains secondary metabolites gene clusters and many functions that suggest possible ecological roles in fish's skin.

Materials And Methods

Vibrio was isolated and cultured in marine agar and incubated at 28°C for 48 h. Genomic DNA was extracted with PowerSoil Kit (MoBio). DNA was used for 300-bp paired-end library preparation with Nextera XT DNA Sample Preparation Kit. The genome sequences were obtained by using MiSeq sequencer (Illumina) as previously described (Walter et al. 2016). Sequences obtained were pre-processed using Prinseq software (v.0.20.4) to remove reads smaller than 35 bp and low-score sequences (Phred 30) (Schmieder & Edwards 2011). Sequence reads were assembled using A5-Miseq software (v.20160825) (Coil et al. 2015). Gene prediction and functional annotation were performed using the Rapid Annotation using Subsystem Technology (RAST) program (Overbeek et al. 2014). In addition, CRISPR arrays were analyzed by using the CRISPRFinder (Grissa et al. 2007).

The closest neighbors of *V. gaelis* A621T were defined based on the results from GTDB-Tk v1.1.0 (Pierre-Alain et al. 2020). The genomes were then uploaded in MiGA (Rodriguez-R et al. 2018) using the whole-genome-based comparison. Through the results from Microbial Genomes Atlas Online (MIGA) the neighbors with at least 70% identity at ANI have been added to the analysis (Table 1). The phylogeny was performed using the concatenated 16S rRNA, *pyrH* gene sequences (accession numbers available in Additional file 1: Table 1). Average amino acid identity (AAI) and average nucleotide identity (ANI) were calculated as previously described (Rodríguez-R & Konstantinidis 2014). Genome-to-genome distance was calculated using the Genome-to-genome distance calculator (GGDC) (Meier-Kolthoff et al. 2013). The parameters used for the delimitation of a new species are less than 95% (AAI/ANI) and less than 70% GGDH with their closest neighbors, in conformity with the method previously described (Thompson et al. 2013; De Vos et al. 2017). Useful *in silico* phenotypes analyses were performed as previously described (Amaral et al. 2014).

Table 1

Genomic and *in silico* phenotypic traits distinguishing *Vibrio gaelis* sp. nov. strain A621^T from closely related Vibrio species. Values of identity (%) are presen 16S rRNA, *pyrH* genes, Concatenated genes, GGD, AAI and ANI between Vibrio species. The *in silico* phenotypic data analyzed included: 1) L-Arabinose; 2) Suc Ornithine; 4) Vogues-Proskauer test; 5) Galactose; 6) Cellobiose; 7) D-Mannitol; 8) Arginine; 9) Trehalose; 10) D-Sorbitol; 11) Indole; 12) M-Inositol; 13) D-Man

Type strains	GGD	ANI	AAI	16S rRNA	pyrH	Concatenated	1	2	3	4	5	6	7	8	9	10	11	12
V. gaelis A621T	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	+	–
V. ponticus CECT 5869T	46.1	90.3	93.6	99.2	95.3	95.3	–	–	–	–	–/+	+	+	–	–	–	+	–
V. panuliri JCM 19500T	30.2	82.9	93.1	97.7	86.1	86.1	–	–/+	–	–	–	+	+	–	–/+	–	+	–
V. rhodolitus G98T	37.6	87.0	89.4	99.5	90.0	90.0	–	–	–	–	–	+	–	–	–	–	+	–
V. taketomensis DSM 106942T	31.0	82.9	87.7	98.0	88.2	88.2	–	+	–	–	–	–/+	–	–	–	–	–	–
V. renipiscarius DCR 1-4-2T	27.5	80.5	80.1	95.6	87.0	87.0	–	–	–/+	–	–/+	–	+	–	–	–	–	–
V. harveyi ATCC 14126T	29.3	81.4	78.8	94.8	87.9	87.9	–	+	+	–	+	+	+	–	+	–	+	–
V. parahaemolyticus ATCC 17802T	28.5	81.0	78.3	95.5	91.5	91.5	+	–	+	–	+	+	+	–	+	–	+	–
V. orientalis ATCC 33934T	27.7	80.3	78.2	95.3	85.8	85.8	–	–	–	–	–	+	–/+	–/+	+	–	+	–
V. tubiashii ATCC 19109T	27.4	80.4	78.1	96.7	88.2	88.2	–	+	–	–	–	–	+	–	–	–	+	–
V. nereis DSM 19584T	27.6	80.1	78.1	96.7	85.3	85.3	–	+	–	–	–	–	+	+	+	–	–/+	–
V. alginolyticus ATCC 17749T	29.2	81.3	77.2	95.5	85.6	85.6	–	+	–/+	+	–/+	+	+	–	+	–	–/+	–
V. natriegens DSM 759T	28.9	81.3	76.9	96.4	90.0	90.0	+	+	–/+	+	–/+	–	+	–	+	–	–/+	+
V. azureus LC2-005T	29.2	80.5	76.7	96.7	85.8	85.8	–	–	–	–	–	–	–	–	–	–	+	–
V. vulnificus ATCC 27562T	27.8	79.9	76.0	95.9	83.8	83.8	–	–/+	+	–	+	+	+	–	+	–	+	–
Enterovibrio baiacu A649T	22.9	77.2	64.3	94.2	77.9	77.9	–	–	+	–	–/+	–	–/+	–	–	–	+	–

The bacterial isolate was obtained from a host with proven toxicity on the skin and, containing TTX, its analogs and precursors (Tonon et al. 2020). The strain A621 previously described (Tonon et al. 2020) as producer of ([M + H] + ion at m/z 320.1088).

Results And Discussion

Reads were assembled in 102 contigs, with an N50 value of 111,428 bp. The estimated genome size is 4,854,211 bp, with a G + C content of 44.5%, and a coverage of 654-fold. In total, 4,431 protein coding sequences, 121 RNAs (112 tRNAs, 1 16S rRNAs, 1 23S rRNAs, and 7 5S rRNAs). The strain had < 90.3% in AAI and > 93.6% ANI, and < 46.1% similarity in GGD, when compared with its closest neighbors and shares 95.3% identity in MLSA with the closest neighbor, *V. ponticus* (Fig. 1 and Table 1). *In silico* phenotypes that differentiate the novel species include presence CHECAR as presented in Table 1. The comparative analyses of *in silico* phenotypic data and Genome-based taxonomic analysis demonstrates that strain A621^T represents a new *Vibrio* species, named *V. gaelis* sp. nov.

In addition, 357 subsystem-related gene sequences were found through RAST. The following stand out: Cofactors, Vitamins, Prosthetic Groups, Pigments (197), Virulence, Disease and Defense 48 (Adhesion was absent), Motility and Chemotaxis 72 (every related to Flagellar motility in Prokaryota). Hypothetical proteins and functions of proteins of unknown function (DUF) by annotation were found (1037) (Omeershffudin & Kumar 2019), indicating that there are still many functions to be thoroughly investigated.

The antiSMASH analyses revealed that this Vibrio may have an important role in the skin mucus of this Tetraodontidae. 4 clusters were found in the antiSMASH analysis related to ectoine, betalactone, redox-cofactor and RiPP-like (Table 2). The presence of LuxR at the same time as the absence of LuxI and LuxM is described as 'eavesdropping'. LuxR is translated into receptors with the role of binding with quorum sensing molecules assembled by other species (Bassler et al. 1994, Bondarev et al. 2013; Case et al. 2008). The reaction response from these receptors can trigger the production of repelling substances increasing the competition or providing the host protection from parasites or pathogens (Bassler et al. 1994, Bondarev et al. 2013; Case et al. 2008). Despite

the presence of several metabolisms related to resistance to toxic compounds and antibiotics, no accessory colonization factors related to adhesion were observed. At the same time, the presence of biosynthesis of vitamin B12, among other vitamins, was found in the genome, and its implications have often been related to the host's health (Agarwal et al. 2019; Bondarev et al. 2013). The differences between an opportunistic microorganism and a symbiont can be in the details. *V. ponticus* has a great number of genomic similarities with this new species, however, it has been shown to be pathogenic for several species of fish in aquaculture (Ina-Salwany et al 2019; Soto-Rodriguez et al 2019). This new specie's potentialities can be tested using it, for example, alternatively in vibrioses caused by *V. ponticus* and others (Ina-Salwany et al. 2019). This demonstrates that *Vibrio gaelis* sp. nov. may confer, directly or indirectly, advantages to the fish's skin but so much more needs to be investigated about these novel bacteria.

Table 2

Vibrio gaelis: gene clusters, subsystems and genes

Gene clusters/Subsystems/Gene	Counts	Relevance to symbiosis	Length (bp)	Literature
Beta-lactone containing protease inhibitor	1	Beta-lactone containing protease inhibitors can counteract the harmful effects of bacterial pathogens and help the host's immune response to eliminate disarmed bacteria.	12.790	Böttcher & Sieber 2009
Ectoine cluster	1	Ectoine has been described as strongly related to the adaptation of vibrios in hypersaline environments and even as possessing thermo protective function. V. anguillarum e V. parahaemolyticus species have been investigated and described as pathogenic for the fish, as well as it's been described as having a role in the skin protection of the human skin. It remains to be seen whether the presence of this function is related to the fitness of the vibrio itself, to benefits for the puffer fish or both.	10.387	Graf et al 2008 - Ma et al 2017 - Ongagna-Yhombi & Boyd 2013
Redox-cofactors such as PQQ	1	Quinone pyrroloquinoline (PQQ) is an omnipresent and the use of probiotics capable of biosynthesizing it has already been appointed as therapy for liver diseases in eukaryotes, since it works as an extraordinarily potent antioxidant. PQQ has been shown to have a related longevity effect and increased resistance to oxidative stress in nematode worms, and also in several eukaryotes. This can bring benefits to the puffer host.	22.130	Wu et al 2016 - Jonscher & Rucker 2019 - Zhu & Klinman 2020
Ribosomally-synthesized and post-translationally modified peptide (RiPP-Like)	1	This RiPP-Like has yet to be investigated, it has a protein of unknown function (DUF692).	10.864	Omeershffudin and Kumar 2019 - Arnison et al 2013
LuxR	8	The presence of LuxR at the same time as the absence of LuxI and LuxM is described as 'eavesdropping'. LuxR is translated into receptors with the role of binding with quorum sensing molecules assembled by other species. The reaction response from these receptors can trigger the production of repelling substances increasing the competition or provide the host protection from parasites or pathogens. Despite presenting several metabolisms related to resistance to toxic compounds and antibiotics, no accessory colonization factors related to adhesion are observed. At the same time, the presence of biosynthesis of vitamin B12, among other vitamins, was found in the genome, and its implications have often been related to the health of the host. The differences between an opportunistic microorganism and a simbiont can be in the details. *V. ponticus* has a great amount of genomic similarities with this new species, however, it has been shown to be pathogenic for several species of fish in aquaculture. This new species's potentialities can be tested using it, for example, alternatively in vibrioses caused by V. ponticus and others.	Not applicable	Azevedo et al 2021 - Agarwal et al 2019 - Ina-Salwany et al 2019 - Soto-Rodriguez et al 2019 - Llewellyn et al 2017 - Bondarev et al 2013 - Case et al 2008 - Bassler et al 1994
LuxI	0			
LuxM	0			
Cobalamin synthesis	12			
Coenzyme B12 biosynthesis	13			
Lipoic acid metabolism	2			
Pyridoxine (B6)	12			
Thiamin biosynthesis (B1)	14			
Biotin biosynthesis (H)	16			
Riboflavin (B2)	23			
Tolerance to colicin E2	1			
Copper homeostasis	12			
Bile hydrolysis	1			
Cobalt-zinc-cadmium resistance	4			
Copper homeostasis: copper tolerance	5			
Fosfomycin resistance	1			
Resistance to fluoroquinolones	2			
Multidrug Resistance Efflux Pumps	5			
Resistance to chromium compounds	1			
Mycobacterium virulence operons	16			
Adhesion	0			

Vibrio gaelis sp. nov. (ga'al from Hebrew, refers to protect). Cells are Gram-negative, facultative aerobic, catalase- and oxidase-positive, 0.5–1.0 μm in diameter after incubation for 48 h at 28°C. Growth occurs at 12 to 35°C in the presence of 1 to 5% NaCl. Optimum growth occurs at 25–30°C in the presence of 3% NaCl. Colonies are circular whole margin and papillary elevation on Marine Agar and TCBS (green colony). This new species is differentiated from its closest neighbor by Galactose utilization (Table 1). *V. gaelis* and *V. ponticus* could express the same phenotype, if the most common phenotyping tests were used, perhaps demonstrating only a small difference in galactose expression, since *V.ponticus* only does not have the GalP_galactose_permease enzyme and could still test positive for this phenotype and *V. gaelis* does not have 5 of the necessary enzymes and would always test negative. It is also possible to notice

differences in the enzymes necessary for the expression of ornithine (absence of 2 enzymes in *V. ponticus* and absence of 3 in *V. gaelis*); for expression of D-Sorbitol (absence of 5 enzymes in *V. ponticus* and absence of 6 in *V. gaelis*); M-inositol (absence of 6 enzymes in *V. ponticus* and absence of 18 in *V. gaelis*). This demonstrates that the use of the *in silico* phenotype is not only useful, it is, above all, more profoundly detailed in the prediction of the phenotype.

V. gaelis A621^T is deposited in the Bacterium Collection of Environmental and Health (CBAS) at Oswaldo Cruz Institute (IOC), FIOCRUZ (Rio de Janeiro, Brazil) (http://cbas. fiocruz.br/) under the accession numbers CBAS 712^T.

The Whole Genome Shotgun Project for *V. gaelis* sp. nov. A621^T was deposited in GenBank under accession number QLYY00000000 (Bioproject: PRJNA476514/SAMN09437379) (https://www.ncbi.nlm.nih.gov/nuccore/QLYY00000000). SRA accession: PRJNA476514.

Declarations

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Conflict of interest

The authors declare no conflicts of interests in the manuscript.

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Figures

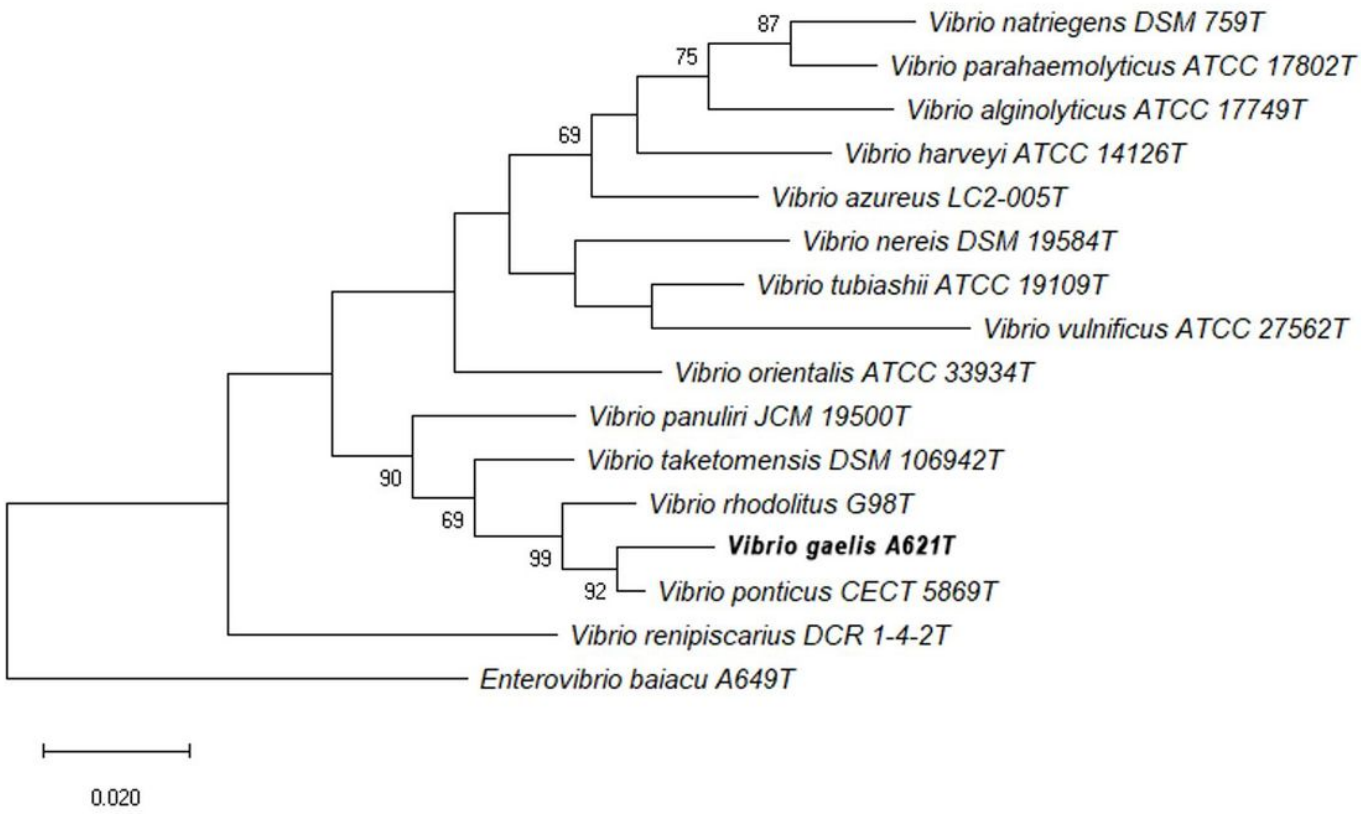

Figure 1

Phylogenetic tree based on 16S rRNA, pyrH, gene sequences, distinguishing Vibrio gaelis sp. nov. strain A621T from closely related Vibrio species. The evolutionary history was inferred by using the Maximum Likelihood method based on the General Time Reversible model (Nei & Kumar, 2000) using MEGA software (version 7.0.26). The sequence of Enterovibrio baiacu A649T was used as outgroup (Azevedo et al., 2020b). Bootstrap values >50 (1,000 resampling) are shown at branching points.

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