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SHORT COMMUNICATION

Isolation, identification, virulence potential and genomic features of *Tenacibaculum piscium* isolates recovered from Chilean salmonids

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Abstract

Tenacibaculum piscium, a gram-negative bacterium isolated from the skin ulcers of sea-farmed fish, has only been described in Norway. In the present study, we examined 16 Chilean *Tenacibaculum* isolates recovered from different organs in moribund and dead Atlantic salmon (*Salmo salar*), Rainbow trout (*Oncorhynchus mykiss*) and Coho salmon (*Oncorhynchus kisutch*) cultured at different fish farms between 2014 and 2018. The present study applied biochemical, phenotypic, fatty acid and whole-genome sequence-based analyses to confirm the taxonomic status of the Chilean isolates. The obtained results are the first to confirm the presence of *T. piscium* in Chile and in Coho salmon, thus extending the recognized geographical and species distribution of this bacterium. Subsequent bath-challenge assays in Atlantic salmon utilizing three *T. piscium* isolates obtained from different hosts resulted in low cumulative mortality (i.e. 0–35%), even after exposure to an unnaturally high concentration of bacterial cells (i.e. $> 10^7$ cells/ml). However, scale loss and frayed fins were observed in dead fish. *In silico* whole-genome analysis detected various genes associated with iron acquisition, encoding of the type IX secretion system and cargo proteins, resistance to tetracycline and fluoroquinolones and stress responses. These data represent an important milestone towards a better understanding on the genomic repertoire of *T. piscium*.

KEYWORDS
Atlantic salmon, Coho salmon, Rainbow trout, tenacibaculosis, virulence

1 | INTRODUCTION

Tenacibaculum species generally present as long rod and/or filamentous gram-negative cells and are widespread in marine environments, commonly adhering to biotic/abiotic surfaces (Olsen et al., 2019; Suzuki et al., 2001). Descriptions from Chile include *Tenacibaculum dicentrarchi* from Atlantic salmon (*Salmo salar*) and red conger eel (*Genypterus chilensis*) (Avendaño-Herrera et al., 2016; Irgang et al., 2017) and *Tenacibaculum maritimum* from turbot (*Scophthalmus maximus*) (Bernardet, 1998; Bridel et al., 2020), Atlantic salmon (Apablaza et al., 2017) and Rainbow trout (*Oncorhynchus mykiss*) (Valdés et al., 2021). *Tenacibaculum finnmarkense* was also confirmed following reclassification of an isolate originally described as *T. dicentrarchi* (Bridel et al., 2018).

Recently recovered nonspeciated *Tenacibaculum* isolates from Chilean Rainbow trout and Coho salmon (*Oncorhynchus kisutch*) were clustered by multilocus sequence analysis typing (Habib et al., 2014) within the *Tenacibaculum* Clades IV (Olsen et al., 2017) and V (Avendaño-Herrera et al., 2020). Members of Clade IV were recently described as *Tenacibaculum piscium* (Olsen et al., 2020), thus indicating the possible presence of this species in Chile.

The only fish species registered, to date, as being affected by *T. piscium*, are Atlantic salmon, Rainbow trout and Corkwing wrasse (*Symphodus melops*), with skin ulcers and eroded fins commonly observed (Olsen et al., 2020). Until now, *T. piscium* had only been described in Norway. The present study applied biochemical, phenotypic and whole-genome sequence-based analyses to confirm the taxonomic status of 16 Chilean isolates as *T. piscium*. The examined isolates were recovered from different organs in moribund and dead Atlantic salmon, Coho salmon and Rainbow trout. Subsequent bath-challenge assays in Atlantic salmon utilizing three *T. piscium* isolates resulted in low cumulative mortality (i.e. 0%–35%). *In silico* whole-genome analysis detected various genes associated with iron acquisition, encoding of the type IX secretion system (T9SS) and cargo proteins, resistance to tetracycline and fluoroquinolones and stress responses. These data represent an important milestone towards a better understanding on the genomic repertoire of *T. piscium*.

2 | MATERIALS AND METHODS

2.1 | Bacterial isolates

The *Tenacibaculum* isolates included in this study are listed in Table 1. This collection includes six Rainbow trout and six Coho salmon isolates previously reported by Avendaño-Herrera et al. (2020) and four additional Atlantic salmon isolates collected during an outbreak of bacterial kidney disease, where the examined fish also displayed clinical signs of tenacibaculosis (e.g. ulcerative lesions on skin and mouth). These four isolates were included due to grouping in cluster IV (Olsen et al., 2017). All isolates were maintained in Criobille tubes (AES Laboratories) at −80°C and routinely grown on *Flexibacter maritimus* medium (FMM) (Pazos et al., 1996) under aerobic conditions for 48–72 h at 18°C and with not more than two subcultures grown from frozen stocks.

2.2 | Biochemical characterization

All isolates were examined using morphological, physiological and biochemical tests (Table 1) (Avendaño-Herrera et al., 2016, 2020; Olsen et al., 2020; Piñeiro-Vidal et al., 2012). Utilization of carbon sources (i.e. D(+)-sucrose, D(−)-ribose, L-proline, D(+)-galactose, D-glucose, L-glutamate and L-tyrosine) was tested on a basal agar medium (0.2 g $NaNO_3$, 0.2 g NH_4Cl, 0.05 g yeast extract, 15 g agar in 1 L sea salts [36 g/L, Sigma]) supplemented with 0.4% (w/v) of each substrate (Suzuki et al., 2001). Furthermore, growth was tested on Columbia base agar (Oxoid) supplemented with laked horse blood (Thermo Fisher Scientific Inc.) and 1.5% NaCl (w/v). All incubations were at 18°C for 10 days. The presence and levels of enzymatic activities were detected using miniaturized API®ZYM systems (bioMérieux) according to the manufacturer's instructions.

2.3 | DNA extraction, sequencing of 16S rRNA and housekeeping genes

DNA was extracted using the InstaGene™ Matrix (Bio-Rad) following the manufacturer's instructions. The 16S rRNA gene was PCR-amplified using primers 27F and 1492R (Lane, 1991). Five housekeeping genes were amplified (*atpD*-816 bp; *fusA*-758 bp; *glyA*-1275 bp; *pgk*-927 bp and *rlmN*-594 bp) (Avendaño-Herrera et al., 2020). All amplicons were sequenced by Macrogen Inc. (Korea).

The resulting 16S rRNA sequences (1,345 bp) and five housekeeping genes were edited and aligned in Geneious Prime v2020.1.1 (https://www.geneious.com), with manual verification in GeneDoc (Nicholas et al., 1997). Substitution saturation was analysed using Xia's method estimated in DAMBE7 (Xia, 2018). Phylogenetic analysis for the 16S rRNA sequences was performed by neighbour-joining in MEGA X (Kumar et al., 2018), and multilocus sequence analysis (MLSA) data were analysed by maximum likelihood and Bayesian Inference in IQ-TREE 1.6.12 (Hoang et al., 2018; Nguyen et al., 2015) and MrBayes v3.0B4 (Ronquist & Huelsenbeck, 2003), respectively.

2.4 | Fatty acid composition analyses

The fatty acid methyl ester compositions of 13 representative isolates were analysed using the Sherlock Microbial Identification System (Sasser, 1990) at the *Colección Española de Cultivos Tipos* (CECT, Spain). All isolates were cultured on marine agar (Difco 2216) incubated at 18°C for 48 h. Fatty acid profiles were obtained using an Agilent 6850 gas chromatographer with the MIDI system using the TSBA6 method (MIDI, 2008). Obtained data were compared to the profiles for *T. piscium* TNO020^T (Olsen et al., 2020).

TABLE 1 Phenotypic characteristics of the Chilean *T. piscium* strains compared to the Norwegian type strain TNO020^T

	Atlantic salmon				Rainbow trout									Coho salmon			Type strain
Characteristics	**IM-14**	**IM-29**	**IM-33**	**IM-44**	**RT-C6**	**RT-C9**	**RT-G4**	**RT-G6**	**RT-G16**	**RT-G19**	**RT-G24**	**RT-G26**	**RT-G28**	**SC-I2**	**SC-I3**	**SC-I4**	**TNO020^T**
Isolation	May 2014	May 2014	May 2014	May 2014	December 2018	December 2018	November 2018	November 2018	November 2018	November 2018	November 2018	November 2018	November 2018	November 2018	November 2018	November 2018	1998
Source	Spleen	Liver	Spleen	Liver	Mouth	Fin	Fin	Operculum	Operculum	Fin	Fin	Fin	Fin	Gill	Lesion	Lesion	Skin ulcer
Geographical region	Magallanes	Magallanes	Magallanes	Magallanes	Los Lagos	Los Lagos	Magallanes	Magallanes	Magallanes	Magallanes	Magallanes	Magallanes	Magallanes	Aysén	Aysén	Aysén	Norway
Fish	3	1	2	1	1	1	1	1	3	3	4	4	4	3	3	5	ni
Morphology	Straight rods	Straight rods	Straight rods	Straight rods	Straight rods	Straight rods	Filamentous rods	Straight rods	Straight rods	Straight rods	Straight rods	Straight rods	Straight rods	Straight rods	Straight rods	Straight rods	Filamentous rods
Motility	Gliding	Gliding	Gliding	Gliding	Gliding	Gliding	Gliding	Gliding	Gliding	Gliding	Gliding	Gliding	Gliding	Gliding	Gliding	Gliding	Gliding
Congo red absoption	+	+	+	w	w	w	+	+	+	w	+	+	w	+	w	w	w
Flexirubin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Blood agar (growth)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Temperature (°C)	1–25	1–25	1–25	1–25	1–25	1–25	1–25	1–25	1–25	1–25	1–25	1–25	1–25	1–25	1–25	1–25	4–25
Seawater (%)	30–100	30–100	30–100	30–100	30–100	30–100	30–100	30–100	30–100	30–100	30–100	30–100	30–100	30–100	30–100	30–100	20–100
pH range	5–10	5–10	5–10	5–10	5–10	5–10	5–9	5–9	5–9	5–9	5–9	5–9	5–9	5–10	5–10	5–10	4–10
Nitrate reduction	–	–	–	–	–	–	–	–	+	+	+	–	–	–	–	+	–
Carbohydrate source																	
L-tyrosine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
L-proline	w	w	w	w	w	w	w	w	w	w	+	w	w	w	w	w	w
D-glucose	–	–	w	w	w	–	w	w	–	w	w	w	w	w	w	w	–
D(+)-galactose	–	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w	–
D(+)-sucrose	w	–	w	w	w	w	w	w	w	w	w	w	w	w	w	w	w
D(−)-ribose	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	w
L-glutamate	+	+	+	+	w	w	w	w	w	w	w	+	+	w	w	w	–
API ZYM																	
Esterase (C4)	–	–	–	–	w	w	w	–	–	–	–	+	w	–	–	–	+
Esterase lipase (C8)	–	–	–	–	–	–	w	–	–	w	–	+	–	–	–	–	–
Cystine arylamidases	+	+	+	+	+	+	+	w	–	–	–	+	+	w	w	w	+
β-glucosidase	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Abbreviations: ng, no growth; ni, not informed; w, weak reaction; +, positive result; −, negative result.

2.5 | Infection challenge assays

The virulence potential of the *Tenacibaculum* isolates was determined by infection trials using 320 Atlantic salmon (≈80 g when obtained, ≈120 g at start of trial). Fish without disease histories and certified free of any Chilean pathogen were obtained from an infection-free, commercial freshwater farm and transported to the FAV S.A. facilities (Puerto Montt, Chile). Prior to transportation, the fish were certified free of any Chilean pathogen (e.g. infectious pancreatic necrosis virus, *Flavobacterium psychrophilum*, *Yersinia ruckeri*, infectious salmon anaemia virus, *Piscirickettsia salmonis*, *Renibacterium salmoninarum*, *T. maritimum*, *T. dicentrarchi* and *Tenacibaculum* spp.). Certification was performed in accordance with existing regulations in Chile; samples were subjected to standard microscopic and bacteriological examinations, as well as PCR analysis in a private diagnostic laboratory recognized by the Chilean National Fisheries and Aquaculture Service (SERNAPESCA).

On arrival to the Experimental Unit of FAV S.A., the fish were randomly distributed between two fiberglass resin tanks containing 750 L of freshwater maintained at 15 ± 1°C and with a water recirculation system (95% recirculation 1.5 times hourly). Fish were fed daily at 1.5% body weight and were maintained in the unit for 60 days prior to the challenge. During the maintenance period, the fish underwent smoltification until obtaining the weight required for the experimental challenge (≈120 g). During smoltification, the fish were maintained at a salinity of 33 ppm. Water temperature and oxygen concentration (8 ppm) were maintained using an automatic monitoring and control system.

For the challenges, the fish were transferred to the maintenance room to the challenge room. The fish were randomly allocated among four groups – three experimental groups (inoculation with isolate IM-14 [from Atlantic salmon], RT-C6 [Rainbow trout] or SC-I2 [Coho salmon]) and one control group (no bacterial inoculation). Groups of 20 Atlantic salmon were randomly distributed among 40 L fiberglass resin tanks. The tanks contained seawater maintained at 33 ppm salinity, 15 ± 1°C, and constant aeration to keep saturation at 70–80%, with oxygen recorded every 30 min. The challenge was performed in duplicate, and two doses were assessed for each isolate. Each inoculum was prepared in marine broth in volumes of 1,500 ml and 500 ml, which corresponded to high and low dose conditions for the conducted challenges. The average bacterial concentrations used for these volumes were ~6.2×10^9 cells/ml for IM-14, ~2.06×10^9 cells/ml for RT-C6 and ~3.11×10^9 for the strain SC-I2, as previously determined by a direct microscopical count using acridine orange (1,000×). Fish were bath-challenged for 5 h with constant aeration.

High (1,500 ml) and low (500 ml) doses were assessed. The final concentrations of bacteria in the tank water were as follows: IM-14, 1.55×10^8 cells/ml (high dose) and 7.75×10^7 cells/ml (low dose); RT-C6, 7.73×10^7 cells/ml (high dose) and 2.58×10^7 cells/ml (low dose) and SC-I2, 7.78×10^7 cells/ml (high dose) and 3.89×10^7 cells/ml (low dose). Control tanks were exposed to bacteria-free marine broth (i.e. two 500 ml tanks and two 1,500 ml tanks).

After 5 h, the treated and control fish were transferred to new tanks (0.3 m^3), and seawater circulation was re-established. Fish were fed at 1.5% body weight daily, and the tank water was 95% recirculated two times hourly. In accordance with previous experimental studies, the trials lasted 7 days. Dead fish were removed daily and examined for clinical signs of tenacibaculosis. To confirm if the inoculated bacterium caused death, smear samples were prepared from external lesions for epifluorescence microscopy with acridine orange (1,000×) to detect long, thin, rod-shaped bacteria. Additionally, samples from external lesions were directly streaked onto FMM plates and incubated at 18°C for up to a week. The suspected *T. piscium* colonies were identified using phenotypic tests.

All procedures involving animals were carried out following the Canadian Council on Animal Care guidelines on the care and use of fish in research, teaching and testing (https://ccac.ca/Documents/Standards/Guidelines/Fish.pdf).

2.6 | Genomic sequencing and genome assembly, annotation and analysis

Genomic DNA was extracted with the Wizard® Genomic DNA Purification Kit (PROMEGA). Genomic DNA from the isolates IM-14, IM-29 and IM-44 were sequenced using an Illumina MiSeq platform with pair-end reads of 300 bp by the Instituto de Medicina Genómica (IMEGEN, Spain). Using the same technology, another seven genomic DNA (isolates RT-C6, RT-C9, RT-G4, RT-G24, SC-I3, SC-I4 and IM-33) were sequenced by Omega Bioservices (Norcross, GA, USA) on an Illumina HiSeqX10 sequencer (Illumina, San Diego, CA, USA) using the pair-end 150 bp run format. These 10 isolates were chosen because they had been recovered from the three species farmed in Chile (i.e. Atlantic salmon, Rainbow trout and Coho salmon). De novo genome assembly included quality control for raw sequences, assembly quality and contig order (Saldarriaga-Cordoba et al., 2021). Contiguous genome sequences were assembled using *T. piscium* TNO020^T as the reference genome (accession number GCA_900239505). Average nucleotide identity (ANI) and *in silico* DNA–DNA hybridization (*is*DDH) analyses were performed (Saldarriaga-Cordoba et al., 2021).

Each genome was annotated with the Rapid Annotation using Subsystem Technology (RAST) server v.2.0 (http://rast.nmpdr.org/; Aziz et al., 2008) and Prokka v1.14.5 (Seemann, 2014). Following annotation, each genome sequence was explored for virulence-related genes (e.g. involved in iron acquisition) using the FeGenie software (Garber et al., 2020). Genes related to antibiotics and toxic compounds were detected using the Comprehensive Antibiotic Resistance Database (Jia et al., 2017) and Subsystem Feature Counts incorporated in RAST annotation. MacSyFinder was used to predict protein-secretion systems (Abby et al., 2014). Finally, each genome was manually curated to identify genes associated with stress response.

2.7 | Strain and genome deposition in public collection and databases

The Atlantic salmon isolates were deposited in CECT as reference strains: IM-14 (CECT 9920), IM-29 (CECT 9921), IM-33 (CECT 9922) and IM-44 (CECT 9923). In addition, the Whole-Genome Shotgun project has been deposited in DDBJ/ENA/GenBank under the BioProject ID PRJNA776342 (BioSamples SAMN22787097 to SAMN22787106). The identifier for the genome assembly of each *T. piscium* isolate is indicated in Supplementary Table 1.

3 | RESULTS AND DISCUSSION

Until the present study, *T. piscium* had only been described in Norway (Olsen et al., 2020), with seven isolates recovered from skin ulcers, eroded tails/fins, normal skin and/or the kidney of Atlantic salmon, Rainbow trout and Corkwing wrasse. In this study, 16 Chilean *Tenacibaculum* isolates were obtained from Atlantic salmon, Rainbow trout and Coho salmon cultured at different fish farms between 2014 and 2018. The Rainbow trout and Coho salmon isolates were recovered mainly from external lesions (Avendaño-Herrera et al., 2020), while the four Atlantic salmon isolates were recovered from internal organs (Table 1). All isolates displayed colony morphology consistent with *Tenacibaculum* species.

All isolates ($n = 16$) were identified as long, straight, gram-negative, gliding rods that were flexirubin negative. Congo red stains were either positive or weakly positive. Colonies on FMM or marine agar presented as flat and circular, with a full pale-yellow edge and nonadherence to the agar. Growth occurred in FMM broth with at least 30% seawater. Abundant growth was observed at 10–25°C, whereas weak growth occurred at temperatures < 5°C. Phenotypically, the Chilean isolates varied in outcomes for carbohydrates utilization, in nitrate, and in the API ZYM test as compared to the Norwegian isolates (Olsen et al., 2020) (Table 1). Such enzymatic heterogenicity has previously been reported for *T. maritimum* isolates of differing origin (Avendaño-Herrera et al., 2004). In instances of weak results, differences might also be explained by the subjective nature of final readings for the API ZYM test.

Two taxonomic methods based on conserved genes established the 16 isolates as *T. piscium*. First, 16S rRNA gene analysis resulted in well-supported clustering (bootstrap support = 84%) with the *T. piscium* type strain TNO020^T, *T. finnmarkense* genomovar *ulcerans* TNO010^T and *T. finnmarkense* genomovar *finnmarkense* TNO006^T (Figure 1a). These relationships were consistent with the high degree of 16S rRNA conservation among *Tenacibaculum* and confirm once again that the 16S rRNA gene is insufficient for discriminating between closely related *Tenacibaculum* species (Avendaño-Herrera et al., 2020; Bridel et al., 2018; Suzuki et al., 2001). Multilocus sequence typing of concatenated housekeeping genes (4,370 bp) using either maximum likelihood or Bayesian Inference resulted in highly similar tree topologies (Figure 1b), including Clades IV and V (Avendaño-Herrera et al., 2020). Interestingly, the MLSA topology slightly differed to that obtained with 16S rRNA, an outcome perhaps resulting from better resolution for MLSA (see Habib et al., 2014). Consequently, the MLSA sequences confirmed phylogenetic relationships between the Chilean isolates and the Norwegian *T. piscium* isolates TNO020^T, TNO063, TNO064 and TNO066 (Olsen et al., 2020). Comparisons between 10 Chilean *T. piscium* isolates (i.e. those previously subjected to genome sequencing) and *T. piscium* TNO020^T through ANI and *is*DDH revealed values of 97.74% < ANI≥ 98.83% and 90.29% < *is*DDH≥ 96.11% (Figure 2). These values are well above the generally accepted species-delineation values of 95–96% (ANI) and 70% (*is*DDH) (Chung et al., 2018; Goris et al., 2007; Meier-Kolthoff et al., 2013). Therefore, all isolates included within clades IV and V belong to *T. piscium*.

The obtained fatty acid profiles most closely resembled that of the *T. piscium* type strain TNO020^T (Olsen et al., 2020). The major components (> 5%) were comprised of a branched chain (Iso-$C_{15:0}$, Anteiso-$C_{15:0}$ and iso-$C_{15.1}$), hydroxylated (iso-$C_{15:0}$ 3-OH and $C_{16:0}$ 3-OH) and summed feature 3 (C16:1w7c/C16:1) fatty acids (Table 2).

Compared to the Chilean *T. dicentrarchi* (Avendaño-Herrera et al., 2016) and *T. maritimum* (Valdes et al., 2021) isolates, the Chilean *T. piscium* isolates appear to present relatively low virulence. The challenge assays had low mortalities (Figure 3), despite the use of higher inocula and longer exposure times (5 h) than in previous studies (Avendaño-Herrera et al., 2016; Valdes et al., 2021). Using inocula of 500 ml and 1,500 ml, mortalities (observed from the first day) were 20% and 22.5% for isolate IM-14 and 22% and 35% for isolate SC-I2, respectively. Isolate RT-C6 presented the lowest/null mortalities (i.e. one death after 3 days). Dead fish suffered scale loss, skin lesions and, for some specimens, tail rot (Figure Supplementary 1). Microscopic examination of skin lesion smears revealed abundant long, thin, rod-shaped gram-negative bacteria (data not shown). Colonies similar to *T. piscium* were recovered on FMM plates from the external lesions of dead fish for IM-14 and SC-I2. Pure and mixed colonies were observed, and microscopic examination of purified colonies revealed gram-negative bacteria presenting a filamentous morphology consistent with *Tenacibaculum*. These colonies were confirmed *T. piscium* through biochemical analyses. No control fish, which were inoculated with the culture medium, showed clinical signs or death during the challenge. These findings suggest that, just as with the Norwegian *Tenacibaculum* Group 2 strain GU124769 (now = TNO020^T) (Olsen et al., 2011), Chilean *T. piscium* isolates are not a primary pathogen and are of minor pathogenic significance.

Whole genome assembly revealed few differences in genome size among the assessed Chilean *T. piscium* (25,19,415 to 26,10,860 bp). All were larger than the type strain (24,52,854 bp). The G + C contents of Chilean isolates (30.61% to 30.81%) were very similar to isolate TNO020^T (30.71%). Aligning with larger genomes, the Chilean isolates presented more coding sequences (2,241 to 2,675) than the type strain (2,213) (Supplementary Table 1). Regarding genomic features, iron acquisition mechanisms are essential virulence factors in other phylogenetically related fish pathogens (e.g. *T. maritimum*; Avendaño-Herrera et al., 2005; Pérez-Pascual et al., 2017 and *T.*

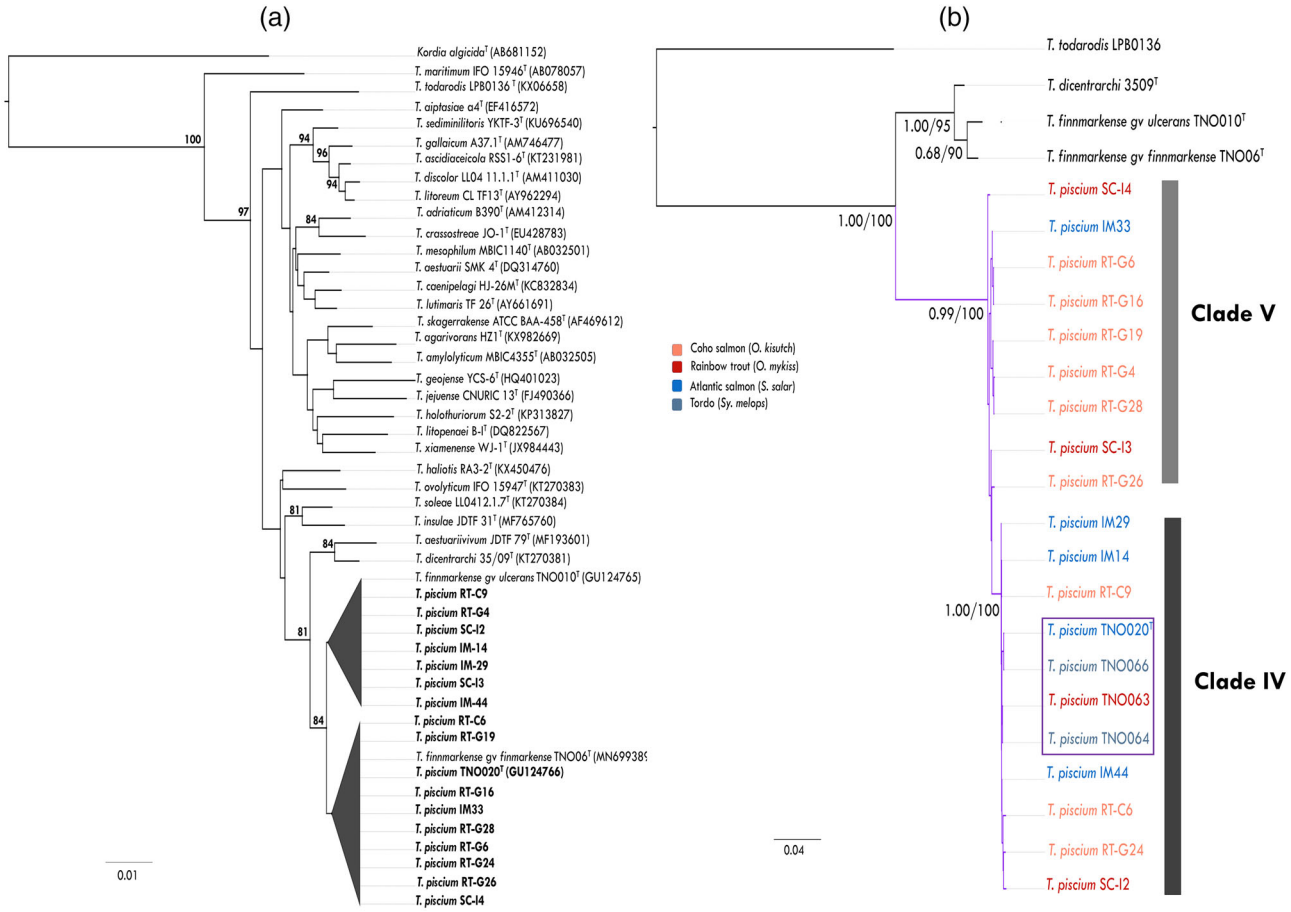

FIGURE 1 (a) Taxonomic positioning of the 16 Chilean *T. piscium* isolates among the 31 *Tenacibaculum* type strains inferred in the MEGA X program by the neighbour-joining algorithm using 1,345 bp of the 16S rRNA gene. The evolutionary distances were computed using the p distance, with a bootstrap of 10,000 replicates. Nodes with a bootstrap support ≥ 80% are indicated in each node. *Kordia algaecide* was used as an external group. (b) Multilocus sequence analyses performed using the maximum-likelihood and information-bottleneck algorithms were based on the five concatenated housekeeping-gene sequences (4,370 bp) of 20 *T. piscium* isolates. Both analyses recovered the same topology. Well-supported nodes are indicated in each node for the maximum-likelihood (bootstrap support ≥ 80%) and information-bottleneck (posteriori probability ≥ 0.95) trees

dicentrarchi; Saldarriaga-Córdoba et al., 2021). The presently studied genome sequences were investigated using FeGenie, a comprehensive software for identifying and characterizing iron-associated genes and operons (Garber et al., 2020). Iron-related protein families (detailed in Supplementary Table 2) were found in all 11 *T. piscium* genomes.

Genome analyses suggest that members of the phylum *Bacteroidetes* use the T9SS to secrete many proteins to the cell surface and beyond (see Barbier et al., 2020). The *T. piscium* genome encodes the core T9SS components, including the cytoplasmic membrane proteins (GldL and GldM), the outer membrane-associated ring-forming proteins (GldN and GldK), the outer membrane pore-forming protein SprA (Sov) and the T9SS plug. Additionally identified were the outer-membrane-associated proteins SprE (PorW), SprF (PorE) and the T9SS-associated PGN_1783-like protein and the outer-membrane proteins PorG, SprT (PorT) and PorV. These proteins are linked to T9SS function in *Flavobacterium johnsoniae* and *Porphyromonas gingivalis*. Proteins secreted by the T9SS have cleavable N-terminal signal peptides required for export across the cytoplasmic membrane via the Sec system. These cargo proteins also have conserved carboxy-terminal domains (CTDs; 60–100 amino acids) that target them for secretion across the outer membrane (Barbier et al., 2020; Kharade & McBride, 2015). Ten T9SS type A (TIGR04183) and six T9SS type B (TIGR04131) CTD-containing proteins were also predicted in the *T. piscium* genomes (Supplementary Table 2). Regarding the stress response (e.g. osmotic, oxidative and periplasmic stress), the Chilean *T. piscium* isolates and type strain displayed the same genes identified in *T. dicentrarchi* (Supplementary Table 2) (Saldarriaga-Córdoba et al., 2021).

Antimicrobial treatments against tenacibaculosis in Chile are primarily with florfenicol, but oxytetracycline is also used (Irgang et al., 2021). Therefore, elucidating determinants of antimicrobial resistance is important. Resistance-related determinants identified included tetracycline (translation elongation factor G-WP_101916661 and tetracycline resistance protein TetQ-WP_101917382), fluoroquinolones (gyrA-WP_193706303 and gyrB-WP_193706307), betalactamases (metal-dependent hydrolases of the beta-lactamase

TNO020^T	SC-I3	RT-C9	IM-29	IM-14	IM-44	IM-33	RT-G24	RT-C6	RT-G4	SC-I4
*is*DDH > 70% same species	95.15	95.99	96.05	96.11	96.04	90.82	90.57	93.12	90.29	90.48
ANI > 96% same species	98.62	98.70	98.71	98.83	98.73	97.94	97.79	97.93	97.74	97.87

FIGURE 2 OrthoANIu, *is*DDH and phylogenetic relationships among 10 Chilean *T. piscium* isolates and the type strain TNO020^T. Phylogeny analysis was performed with whole-genome sequence data in the Reference Sequence Alignment-based Phylogeny builder inferred via PhyML (Bertels et al., 2014). Blue dots indicate a bootstrap support ≥ 98%

superfamily I-WP_101916599) and triclosan and isoniazid (fabV-WP_101916498). Excepting *fabV*, all of these genes were previously identified in *T. dicentrarchi* (Saldarriaga-Córdoba et al., 2021). RAST analysis also identified genes associated with resistance to toxic compounds (e.g. copper and cobalt/zinc/cadmium; Supplementary Table 2). Metal-responsive transcriptional regulators play pivotal roles in metal uptake, pumping out, sequestration and oxidation/reduction to a less toxic status (Jung & Lee, 2019). The *T. piscium* genome encoded for a multidrug and toxic compound extrusion transporter (WP_101917373), which expels antibiotics out of the cell. Almost all such transporters recognize fluoroquinolones; however, acriflavine and aminoglycosides are also good substrates (Kuroda & Tsuchiya, 2009).

In conclusion, the present report is the first to confirm the presence of *T. piscium* in Chile, thus extending its recognized geographical distribution. The obtained findings also indicate that *T. piscium* presents low pathogenicity for Atlantic salmon, even after exposure to an unnaturally high concentration of bacterial cells (i.e. over 10^7 cells/ml). However, scale loss and frayed fins were observed in dead fish. Further reported herein are the draft genomes of 10 Chilean field isolates. Genome analysis identified genes associated with virulence, including iron acquisition mechanisms, encoding of the T9SS, resistance to tetracycline and fluoroquinolones and involved in stress response that likely contribute to niche adaptation and disease development.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

TABLE 2 Fatty acid profile of the Chilean *T. piscium* isolates and the Norwegian type strain TN0020^T

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Straight chain														
$C_{13:1}$ (12–13)		0.54			0.30	0.76	0.68	0.52	0.58	0.59	0.38	0.47	0.52	
$C_{14:0}$	1.09	1.10	1.00	1.11	1.07	1.07	1.56	1.11	1.06	1.07	1.33	1.18	1.16	1.6
$C_{15:0}$														2.2
$C_{16:0}$	1.79	1.59	1.88	1.64	2.53	1.68	2.71	2.20	1.96	2.52	2.57	2.55	1.93	
Branched chain														
Iso-$C_{13:0}$	0.39	0.36	0.43	0.42	0.39	0.47	0.54	0.51	0.55	0.52	0.77	0.48	0.84	0.5
Anteiso $C_{13:0}$			0.19	0.25	0.26			0.25	0.20	0.25	0.25	0.24	0.24	
Iso-$C_{14:0}$	1.27	1.20	2.15	1.42	1.50	1.08	1.24	1.79	1.51	1.44	1.24	1.05	1.58	2.4
Iso-$C_{15:0}$	**13.62**	**13.78**	**12.06**	**13.02**	**12.65**	**15.07**	**16.38**	**13.11**	**13.54**	**13.78**	**16.06**	**15.03**	**14.63**	**11.1**
Anteiso $C_{15:0}$	**10.36**	**11.40**	**12.93**	**10.15**	**13.16**	**12.30**	**10.74**	**14.25**	**14.33**	**13.88**	**11.46**	**13.28**	**12.15**	**14.0**
Iso-$C_{15:1}$	**8.62**	**8.73**	**9.85**	**8.50**	**8.49**	**9.37**	**10.27**	**10.22**	**9.82**	**10.73**	**9.97**	**9.25**	**10.17**	**9.6**
Anteiso $C_{15:1}$	1.17	1.19	1.77	1.10	1.49	1.48	1.40	1.99	1.85	1.92	1.28	1.38	1.60	2.1
Iso-$C_{16:0}$	0.56	0.58	0.67	0.62	0.72	0.57	0.61	0.71	0.66	0.81	0.57	0.54	0.72	1.4
Iso-$C_{16:1}$	1.91	1.74	2.33	2.13	1.96	1.66	1.60	2.41	2.25	2.20	1.65	1.76	2.08	3.3
Unsaturated														
$C_{15:1}$ *ω6c*	4.03	4.13	3.87	4.51	3.12	3.57	1.94	3.00	3.50	2.66	2.25	3.17	3.54	3.2
$C_{16:1}$ *ω5c*	2.39	2.64	2.18	2.51	2.06	2.73	2.81	2.28	2.40	2.20	2.63	2.85	2.48	2.8
$C_{17:1}$ *ω6c*	3.11	2.91	2.04	3.40	2.41	2.95	1.31	1.75	1.67	1.87	1.42	2.52	1.71	2.4
$C_{18:1}$ *ω5c*	0.64	0.68	0.54	0.61	0.53	0.68	0.51	0.56	0.55	0.61	0.56	0.62	0.55	
$C_{18:1}$ *ω9c*												0.71		
Hydroxylated														
Iso-$C_{15:0}$ 3-OH	**15.10**	**14.31**	**14.55**	**14.88**	**13.88**	**12.34**	**12.11**	**12.33**	**12.04**	**11.53**	**12.03**	**9.63**	**13.79**	**8.2**
$C_{15:0}$ 2-OH	0.77	0.82	0.88	0.76	0.91	0.69	0.55	0.93	0.86	0.80	0.66	0.76	0.80	0.7
$C_{15:0}$ 3-OH	2.07	1.95	1.66	2.12	1.66	2.03	1.23	1.64	1.60	1.68	1.11	1.66	1.71	Tr
Iso-$C_{16:0}$ 3-OH	4.02	3.79	**5.45**	4.19	4.50	2.76	2.78	4.23	3.69	3.58	2.91	2.63	3.30	4.0
$C_{16:0}$ 3-OH	**6.38**	**6.09**	**6.48**	**6.11**	**6.67**	**5.10**	**6.32**	**5.79**	**5.12**	5.37	6.17	4.81	4.47	4.4
Iso-$C_{17:0}$ 3-OH	3.21	3.18	3.00	2.88	3.09	3.28	3.11	2.78	2.85	3.29	3.23	3.02	2.69	1.6
$C_{17:0}$ 2-OH	0.66	0.77	0.76	0.57	0.92	0.71		0.72	0.82	0.80	0.61	0.72	0.55	
$C_{18:0}$ 10-methyl TBSA	0.72	0.67	0.61	0.76	0.57	0.45		0.32	0.31	0.30		0.38		
Summed feature 3[a]	**15.58**	**15.38**	**12.71**	**15.94**	**14.66**	**16.39**	**18.86**	**14.03**	**15.73**	**14.90**	**18.20**	**16.98**	**16.14**	**17.7**
Summed feature 9[a]	0.55	0.48		0.41	0.49	0.78	0.72	0.58	0.54	0.70	0.69	0.67	0.66	

Note: Strains: From Atlantic salmon origin: 1, IM-14; 2, IM-29; 3, IM-33 and 4, IM-44; Rainbow trout: 5, RT-C6; 6, RT-C9; 7, RT-G4; 8, RT-G6; 9, RT-G24; 10, RT-G26; and 11, RT-G28 and Coho salmon: 12, SC-I2; 13, SC-I3. Data for 14, *T. piscium* TN0020^T were retrieved from Olsen et al. (2020). Major fatty acids (> 5%) are shown in bold.

[a]Summed feature 3 comprises $C_{16:1}$*w7c*/$C_{16:1}$ *w6c* and Summed feature 9 comprises for $C_{17:1}$-iso *w9c*/$C_{16:0}$ 10-methyl.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available on request from the corresponding author. The genome sequencing data that support the findings of this study are available for free download in the GenBank.

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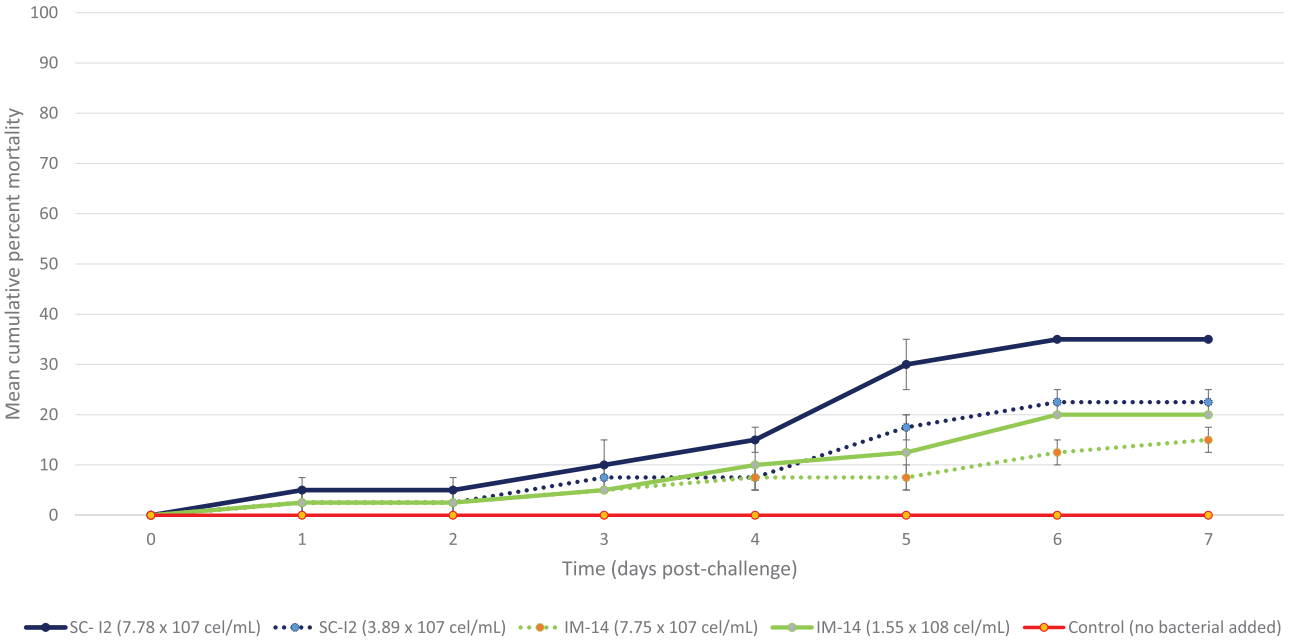

FIGURE 3 Mean cumulative per cent mortalities of Atlantic salmon immersion challenged with an inoculum of 500 ml (low dose) or 1,500 ml (high), respectively, containing bacterial concentrations of 7.75×10^7 cells/ml and 1.55×10^8 cells/ml for IM-14 (Atlantic salmon) and 3.89×10^7 cells/ml and 7.78×10^7 cells/ml for SC-I2 (Coho salmon). All treatments were performed in duplicate, and two control tanks were immersed in bacteria-free marine broth. Specimens challenged with RT-C6 (Rainbow trout), respectively, presented no and a single mortality at doses of 2.58×10^7 cells/ml and 7.73×10^7 cells/ml

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