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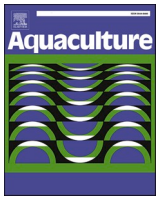

Genomics and serotyping of *Tenacibaculum maritimum* outbreak isolates from Australia and New Zealand for guided vaccine development and stewardship

O. Rudenko [a,*], C. Angelucci [b], K. Kumanan [c,d], L. Delisle [c,d], J. Carson [e], R.N. Morrison [b], K.S. Hutson [c,d], A.C. Barnes [a]

[a] School of the Environment and Centre for Marine Science, The University of Queensland, Queensland, Australia
[b] Centre for Aquatic Animal Health and Vaccines, Department of Natural Resources and Environment Tasmania, Tasmania, Australia
[c] Cawthron Institute, Nelson, New Zealand
[d] Centre for Sustainable Tropical Fisheries and Aquaculture, College of Science and Engineering, James Cook University, Townsville, Queensland, Australia
[e] Carson BioConsulting, Launceston, Tasmania, Australia

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ABSTRACT

Tenacibaculum maritimum is a ubiquitous marine bacterium with a remarkable ability to adhere to and form biofilm structures on abiotic and biotic surfaces including the skin and mucus of various marine fish species. Excessive colonisation of the fish epidermal surface is often associated with tenacibaculosis, a life-threatening skin infection that can progress to ulcerative lesions. Tenacibaculosis causes ongoing losses to finfish aquaculture worldwide, including the Atlantic salmon (*Salmo salar*) industry in Tasmania, Australia, and the King (Chinook) salmon (*Oncorhynchus tshawytscha*) industry in New Zealand, with no effective vaccine available. In this study we provide an evidence base for vaccine development by linking genomic analysis with antibody-based serotyping. We assembled and curated thirty-six complete genomes and thirty-six contig-level genomes of isolates from Tasmania and New Zealand, as well as three genomes of the overseas strains – Baxa lyl-1 (contig-level), NCIMB 2158 (complete), and NCIMB 2154^T type strain (complete). Core genome phylogenetic analysis indicated a high degree of geographic endemism and largely independent evolution of *T. maritimum* in Australia and New Zealand. Two main O-antigen gene cluster (O-AGC) types were identified in each location: type 3–2 was predominant in Tasmania, type 3–0 was predominant in New Zealand, while type 2–1 was the second most abundant type in both Tasmania and New Zealand. O-AGC type 3–2 appears to be endemic to Oceania, and one of the distinct phylogenetic lineages harbouring this O-antigen type (clade C1) appears to be endemic to Tasmania. Somewhat surprisingly, O-AGC types were highly conserved within phylogenetic clades and/or subclades. In the case of Tasmania, there was a partial concordance between O-AGC typing and antibody-based serotyping. Importantly, variation in serotyping results within O-AGC type 3–2 isolates was clearly linked to phylogenetic clustering. In the case of New Zealand, antibody serotyping did not distinguish between O-AGC types. We hypothesise this may be due to high amount of a colanic acid (CA) capsule or similar exopolysaccharide and its binding to the LPS core. Stress-inducible CA capsule is critical for biofilm formation in other bacterial species, and here we report for the first time the putative CA exopolysaccharide cluster (K-AGC) in *T. maritimum* which may aid more rigorous typing and vaccine development.

1. Introduction

Tenacibaculum maritimum is a marine gram-negative filamentous bacterium of the family *Flavobacteriaceae*. Originally identified as hatchery pathogen of black and red sea bream, *Acanthopagrus schlegelii* and *Pagrus major,* respectively (Masumura and Wakabayashi, 1977), *T. maritimum* is the causative agent of tenacibaculosis which presents as cutaneous erosion such as ulcerative skin lesions, mouth erosion, frayed fins and tails which may be fatal. A wide range of commercially important marine fish species are susceptible to *T. maritimum* infection,

* Corresponding author.
E-mail address: o.rudenko@uq.edu.au (O. Rudenko).

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including Atlantic salmon, *Salmo salar* (Australia, Norway, Chile and Canada), sea bass, *Dicentrarchus labrax* (France), turbot, *Scophthalmus maximus,* and sole, *Solea solea* (Spain), and Chinook (king) salmon, *Oncorhynchus tshawytscha* (New Zealand) (Avendaño-Herrera et al., 2006; Kumanan et al., 2024; Mabrok et al., 2022). In the late 1980s *T. maritimum* was identified (as *Flexibacter sp.*) in diseased farmed rainbow trout and Atlantic salmon in Tasmania (Carson et al., 1993; Schmidtke et al., 1991) and farmed Chinook salmon in New Zealand (Boustead, 1989). Without effective commercially available vaccines, the salmonid aquaculture industries in Oceania continue to have limited preventative capability against tenacibaculosis.Therefore, insights into local phylogenetic and antigenic diversity are required for guided development and stewardship of vaccines.

Robust identification of antigenic groups in the *T. maritimum* population is critical for broad spectrum vaccine development. Multiple studies employing antibody-based serotyping methods have demonstrated antigenic heterogeneity in *T. maritimum* populations (Avendaño-Herrera et al., 2004a; Avendaño-Herrera et al., 2005; Castro et al., 2007; Fernández-Álvarez and Santos, 2018; Ostland et al., 1999; Pazos, 1997; Piñeiro-Vidal et al., 2007), although serotype homogeneity has been observed in local *T. maritimum* isolates in Japan (Wakabayashi et al., 1984) and Turkey (Yardimci and Timur, 2016). An O-antigen serotyping scheme consisting of four serogroups is currently considered to represent the global *T. maritimum* population (Fernández-Álvarez et al., 2018; Mabrok et al., 2022). Assigning isolates to these four serogroups is challenging due to inconsistencies between laboratories when using antibody-based serotyping, for example for type strain NCIMB 2154^T (Avendaño-Herrera et al., 2004a; Pazos, 1997). Consequently, there is an ongoing effort to establish a robust molecular serotyping scheme for *T. maritimum.*

Molecular typing of *T. maritimum* using ribosomal RNA (rRNA) fragmentation (Pazos, 1997), randomly amplified polymorphic DNA (RAPD) (Avendaño-Herrera et al., 2004b), repetitive extragenic palindromic PCR (REP-PCR), or enterobacterial repetitive intergenic consensus sequence PCR (ERIC-PCR) (Fernández-Álvarez et al., 2018) did not correlate to four serotypes. However, the degree of association between molecular and antibody serotyping is determined by the choice of genetic loci. Whole genome data from diverse isolates has enabled development, evaluation, and implementation of molecular serotyping approaches in human and veterinary medicine. For example, *Salmonella enterica*, serotypes predicted from raw NGS reads using SeqSero antigen typing scheme targeting O- and H-antigen genes, and serotypes predicted by Multilocus Sequence Typing (MLST) targeting several housekeeping genes, showed 98% and 95% correlation with antisera-based serotyping, respectively. Accuracy of prediction increased to $>$ 99% when results of the MLST and SeqSero molecular typing methods were combined (Banerji et al., 2020). For *T. maritimum,* a MLST scheme based on variation in seven core genome loci has been established (Habib et al., 2014), and MLST data for the species are available (see https://pubmlst.org/tenacibaculum/; (Jolley et al., 2018). Recently, a molecular serotyping scheme based on the O-antigen gene cluster (O-AGC) was also developed for global *T. maritimum* isolates where four major molecular O-ACG types corresponded with four antiserum-based serotypes (Lopez et al., 2022).

The aim of this study was to provide an evidence base to facilitate the development of effective vaccines against *T. maritimum* in Australia and New Zealand by linking comparative genomics and antisera-based serotyping of isolates collected from clinically diseased farmed fish. We generated comprehensive high-quality genomic data including multiple complete genomes, determined phylogenetic relationship using core genome polymorphisms, applied MLST and O-AGC typing, evaluated the (dis)concordance of O-AGC typing with antiserum-based setyping results, and identified K-AGC, the K-antigen genetic cluster encoding colanic acid exopolysaccharide antigen.

2. Methods

2.1. Bacterial isolates and culturing

A total of 75 *T. maritimum* isolates were used in the study (Table 1). Forty-one were held at the Tasmanian Collection of Fish Bacteria (TCFB) at the Department of Natural Resources and Environment, Tasmania. These isolates were collected through Tasmanian diagnostic submissions following the standard isolation methods as described (Handlinger, 2008; Schmidtke et al., 1991). TCFB isolates were collected between 1989 and 2018 from Atlantic salmon (n = 31), rainbow trout, *Oncorhynchus mykiss* (n = 4), striped trumpeter, *Latris lineata* (n = 2), and greenback flounder, *Rhombosolea tapirina* (n = 2), in South-Eastern Tasmania, and 2 isolates from Atlantic salmon in Northern Tasmania (Table 1). Three strains obtained from overseas (NCIMB 2154^T type strain, NCIMB 2158, Baxa lyl-1) and held at TCFB were also included. The remaining thirty-one isolates were obtained from farmed Chinook (King) salmon in New Zealand in 2020- 21 and held at the Cawthron Culture Collection of Microorganisms (CCCM) (Kumanan et al., 2024).

Frozen *T. maritimum* isolates were revived from −80°C on modified Marine Sheih's agar (MSA) comprised of 0.5% *w/v* bacteriological peptone, 0.05% w/v yeast extract, 0.001% w/v sodium acetate, 0.01% w/v sodium pyruvate, 0.001% w/v citric acid, 10% v/v distilled water and 90% v/v aged natural seawater, pH adjusted to 7.4–7.8, 1.5% v/v bacteriological agar (Van Gelderen et al., 2010). Species identity was confirmed by amplification and sequencing almost entire 16S rDNA gene using 27F EubB and 1492R primers, and its fragment using 503F and 907R primers (Lane, 1991). The resulting four sequences were assembled into contiguous open reading frames for each isolate with GeneStudio™ (Version 2.2.0.0). Purity of DNA samples was judged by inspection of sequence chromatograms. Broth cultures were grown in Marine Sheih's broth (MSB; as MSA but omitting 1% v/v bacteriological agar) at 22°C with vigorous shaking. In the case of TCFB isolates, the pH of MSB was adjusted to 7.4 and cultures grown at 270 rpm on Labwit ZWYR 2102C shaker. In the case of CCCM isolates, the pH of MSB was adjusted to 7.8 and cultures grown at at 180 rpm on Ratek orbital shaker.

2.2. DNA extraction and sequencing

2.2.1. Illumina

Illumina sequencing was performed on genomic DNA (gDNA) extracted from all 75 isolates. gDNA was extracted using the Qiagen DNeasy Blood and Tissue Kit (Qiagen) or Quick DNA Miniprep Plus kit (ZymoResearch) for the TCFB and CCCM isolates, respectively. One nanogram of purified gDNA for each isolate was used to prepare Nextera XT libraries barcoded for a multiplexed sequencing run using medium output on the Illumina NextSeq500 instrument at the Ramaciotti Centre for Genomics, University of New South Wales, Sydney (TCFB isolates) or Australian Centre of Ecogenomics, University of Queensland, Brisbane (CCCM isolates).

2.2.2. PacBio

gDNA of isolate TCFB 4754 was extracted using the cetyltrimethylammonium bromide (CTAB) method (Wilson, 2001) and subjected to PacBio sequencing. gDNA sequencing libraries were prepared using the Template Prep Kit 1.0-SPv3 (PacBio, 100–991-900) according to the standard protocol for multiplexed microbial SMRTbell libraries for the PacBio Sequel system (PacBio, Part # 101–489-000 v2). A total of 1.5 μg of gDNA was sheared to ~10 kb using Covaris g-TUBEs, purified with AMPure PB beads, diluted to 75 ng/μL 13.2 μL (990 ng), subjected to Exonuclease VII digestion, followed by a DNA end-repair reaction, and purified with AMPure PB beads, and barcoded by ligation of the adapters (PacBio, 101–081–300/101–081–400). The barcoded samples were pooled into 4-plex reactions in ratios according to the PacBio Microbial Multiplexing Calculator (PacBio), digested with Exonuclease III

Table 1
List of the 75 isolates *T. maritimum* isolates included in this study.

Isolate ID	Host	Year	Origin	Genome accession, sequencing platform/s	Clade	MLST	O-AGC
NCIMB 2154^{T}	Red sea bream (*Pagrus major*)	1977	Japan	CP138495 Nanopore/Illumina	B	1	1–0
NCIMB 2158	Dover sole (*Solea solea*)	1981	Scotland	CP138494 Nanopore/Illumina	B	10	2–1
Baxa lyl-1	Japanese flounder (*Paralichthys olivaceus*)	1986	South Korea	JAZGLG000000000 Illumina	B	25	1–0
TCFB 0022 DPIF 89/4986–1	Atlantic salmon (*Salmo salar*)	1989	South East Tasmania	CP137733 Nanopore/Illumina	B-anz3	**126**	2–1
TCFB 0023 DPIF 89/4983–3	Atlantic salmon (*Salmo salar*)	1989	South East Tasmania	JAZGLL000000000 Illumina	B-anz4	19	**3–2**
TCFB 0024 DPIF 89/4942–2	Atlantic salmon (*Salmo salar*)	1989	South East Tasmania	JAZGLK000000000 Illumina	C1	**131**	**3–2**
TCFB 0418 DPIF 99/2558–2	Atlantic salmon (*Salmo salar*)	1989	South East Tasmania	JAZGLF000000000 Illumina	B-anz4	19	**3–2**
TCFB 0492 DPIF 89/0329–5	Atlantic salmon (*Salmo salar*)	1989	South East Tasmania	JAZGLD000000000 Illumina	B-anz4	22	2–1
TCFB 0509 DPIF 89/1002	Atlantic salmon (*Salmo salar*)	1989	South East Tasmania	JAZGLC000000000 Illumina	B-anz5	21	**3–0**
TCFB 0512 DPIF 89/1579-2S	Atlantic salmon (*Salmo salar*)	1989	South East Tasmania	JAZGLA000000000 Illumina	B-anz4	22	2–1
TCFB 0543 DPIF 89/5118-1sk	Atlantic salmon (*Salmo salar*)	1989	South East Tasmania	JAZGKY000000000 Illumina	B-anz5	21	**3–0**
TCFB 0648 DPIF 89/4762	Atlantic salmon (*Salmo salar*)	1989	South East Tasmania	CP137732 Nanopore/Illumina	B-anz4	**127**	2–1
TCFB 0482 DPIF 89/0235–1	Rainbow trout (*Oncorhynchus mykiss*)	1989	South East Tasmania	JAZGLE000000000 Illumina	B-anz5	21	**3–0**
TCFB 0510 DPIF 89/1288–8	Rainbow trout (*Oncorhynchus mykiss*)	1989	South East Tasmania	JAZGLB000000000 Illumina	B-anz4	22	2–1
TCFB 0518 DPIF 89/3001–2	Striped trumpeter (*Latris lineata*)	1989	South East Tasmania	JAZGKZ000000000 Illumina	C1	24	**3–2**
TCFB 0019 DPIF 90/1123	Atlantic salmon (*Salmo salar*)	1990	South East Tasmania	JAZGLM000000000 Illumina	B-anz4	19	**3–2**
TCFB 0031 DPIF 90/1445	Atlantic salmon (*Salmo salar*)	1990	South East Tasmania	JAZGLJ000000000 Illumina	B-anz4	19	**3–2**
TCFB 0187 DPIF 91/0126	Atlantic salmon (*Salmo salar*)	1991	South East Tasmania	JAZGLI000000000 Illumina	B-anz4	19	**3–2**
TCFB 0188 DPIF 91/0247	Atlantic salmon (*Salmo salar*)	1991	South East Tasmania	JAZGLH000000000 Illumina	B-anz2	**132**	2–1
TCFB 0638 DPIF 95/6339 -	Greenback flounder (*Rhombosolea tapirina*)	1995	South East Tasmania	JAZGKX000000000 Illumina	C1	**133**	**3–2**
TCFB 0639 DPIF 95/6339 —B	Greenback flounder (*Rhombosolea tapirina*)	1995	South East Tasmania	JAZGKW000000000 Illumina	C1	**133**	**3–2**
TCFB 0663 DPIF 96/0457–4	Atlantic salmon (*Salmo salar*)	1996	South East Tasmania	JAZGKV000000000 Illumina	B-anz3	**128**	2–1
TCFB 0759 DPIF 98/3186	Atlantic salmon (*Salmo salar*)	1998	South East Tasmania	JAZGKU000000000 Illumina	C1	**134**	**3–2**
TCFB 0814 DPIF 99/2558–2	Rainbow trout (*Oncorhynchus mykiss*)	1999	South East Tasmania	JAZGKR000000000 Illumina	C2	**136**	**4–0**
TCFB 0809 DPIF 00/0400–3	Atlantic salmon (*Salmo salar*)	2000	South East Tasmania	JAZGKT000000000 Illumina	C1	**134**	**3-2**$^{M2–1}$
TCFB 0811 DPIF 00/0422–3	Atlantic salmon (*Salmo salar*)	2000	South East Tasmania	JAZGKS000000000 Illumina	B-anz2	**135**	2–1
TCFB 0813 DPIF 00/0813	Atlantic salmon (*Salmo salar*)	2000	South East Tasmania	CP137731 Nanopore/Illumina	B-anz3	**128**	2–1
TCFB 0853 DPIF 00/1793–1	Atlantic salmon (*Salmo salar*)	2000	South East Tasmania	JAZGKQ000000000 Illumina	C1	**134**	**3–2**
TCFB 0856 DPIF 00/3280	Rainbow trout (*Oncorhynchus mykiss*)	2000	Northern Tasmania	JAZGKP000000000 I Illumina	B-anz3	**128**	2–1
TCFB 1813 DPIF 02/1125	Atlantic salmon (*Salmo salar*)	2002	South East Tasmania	JAZGKN000000000 Illumina	B-anz4	20	**3–2**
TCFB 1513 DPIF 03/0587-4sk	Striped trumpeter (*Latris lineata*)	2003	South East Tasmania	JAZGKO000000000 Illumina	B	**137**	1–0
TCFB 2854 DPIF 09/0160-4 m	Atlantic salmon (*Salmo salar*)	2009	Northern Tasmania	JAZGKM000000000 Illumina	B-anz2	**138**	2–1
TCFB 2947 DPIF 10/0393-4sk	Atlantic salmon (*Salmo salar*)	2010	South East Tasmania	JAZGKL000000000 Illumina	B-anz4	19	**3–2**

(*continued on next page*)

Table 1 (*continued*)

Isolate ID	Host	Year	Origin	Genome accession, sequencing platform/s	Clade	MLST	O-AGC
TCFB 3064 DPIF 13/0033-3Sk	Atlantic salmon (*Salmo salar*)	2013	South East Tasmania	JAZGKK000000000 Illumina	B-anz4	**129**	3–1
TCFB 3289 DPIF 14/1817	Atlantic salmon (*Salmo salar*)	2014	Northern Tasmania	JAZGKJ000000000 Illumina	B-anz3	**139**	2–1
TCFB 4536 DPIF 17/1647–6	Atlantic salmon (*Salmo salar*)	2017	South East Tasmania	JAZGKI000000000 Illumina	B-anz4	20	**3–2**
TCFB 4538 DPIF 17/1876–2	Atlantic salmon (*Salmo salar*)	2017	South East Tasmania	JAZGKH000000000 Illumina	B-anz4	**130**	**3–2**
TCFB 4540 DPIF 17/1957–8	Atlantic salmon (*Salmo salar*)	2017	South East Tasmania	CP137730 Nanopore/Illumina	B-anz4	20	**3–2**
TCFB 4542 DPIF 17/2068–2	Atlantic salmon (*Salmo salar*)	2017	South East Tasmania	JAZGKG000000000 Illumina	B-anz2	**140**	2–1
TCFB 4574 DPIF 17/3739–2	Atlantic salmon (*Salmo salar*)	2017	South East Tasmania	CP137729 PacBio/Illumina	C2	**141**	**4–0**
TCFB 4579 DPIF 17/3964–10	Atlantic salmon (*Salmo salar*)	2017	South East Tasmania	JAZGKF000000000 Illumina	C2	**141**	**4–0**
TCFB 4607 DPIF 18/0381–3	Atlantic salmon (*Salmo salar*)	2018	South East Tasmania	JAZGKE000000000 llumina	B-anz4	19	**3–2**
TCFB 4635 DPIF 18/1257–8	Atlantic salmon (*Salmo salar*)	2018	South East Tasmania	JAZGKD000000000 llumina	C1	**142**	3–1
TCFB 4646 DPIF 18/1025–4	Atlantic salmon (*Salmo salar*)	2018	South East Tasmania	JAZGKC000000000 llumina	B-anz4	**130**	**3–2**
CCCM 001	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Waihinau, New Zealand*	CP137271 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 004	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Waihinau, New Zealand *	CP137270 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 005	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Waihinau, New Zealand *	CP137269 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 006	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Waihinau, New Zealand *	CP137268 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 010	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Waihinau, New Zealand *	CP137267 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 013	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Forsyth, New Zealand *	CP137266 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 014	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Forsyth, New Zealand *	CP137265 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 015	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Forsyth, New Zealand *	CP137264 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 017	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Forsyth, New Zealand *	CP137263 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 019	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Forsyth, New Zealand *	CP137262 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 020	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Forsyth, New Zealand *	CP137261 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 034	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Forsyth, New Zealand *	CP137260 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 035	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Forsyth, New Zealand *	CP137259 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 038	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Forsyth, New Zealand *	CP137258 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 039	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Forsyth, New Zealand *	CP137257 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 085	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Waihinau, New Zealand *	CP137256 Nanopore/Illumina	B-anz5	**186**	**3–0**
CCCM 101	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Clay Point, New Zealand [Y]	CP137255 Nanopore/Illumina	B-anz5	21	**3–0**
CCCM 102	Chinook salmon (*Oncorhynchus tshawytscha*)	2020	Waihinau, New Zealand *	CP137254 Nanopore/Illumina	B-anz4	19	**3–2**
CCCM 104	Chinook salmon (*Oncorhynchus tshawytscha*)	2021	Te Pangu, New Zealand [Y]	CP137253 Nanopore/Illumina	B-anz5	21	**3–0**
CCCM 105	Chinook salmon (*Oncorhynchus tshawytscha*)	2021	Ruakaka, New Zealand [Y]	CP137252 Nanopore/Illumina	B-anz5	21	**3–0**
CCCM 106	Chinook salmon (*Oncorhynchus tshawytscha*)	2021	Kopaua, New Zealand *	CP137251 Nanopore/Illumina	B-anz5	**187**	**3–0**
CCCM 108	Chinook salmon (*Oncorhynchus tshawytscha*)	2021	Ngāmahau, New Zealand [Y]	CP137250 Nanopore/Illumina	B-anz5	21	**3–0**
CCCM 123	Chinook salmon (*Oncorhynchus tshawytscha*)	2021	Ruakaka, New Zealand [Y]	CP137249 Nanopore/Illumina	B-anz4	19	**3–2**
CCCM 125	Chinook salmon (*Oncorhynchus tshawytscha*)	2021	Waitata, New Zealand *	CP137248 Nanopore/Illumina	B-anz1	**178**	2–1
CCCM 127	Chinook salmon (*Oncorhynchus tshawytscha*)	2021	Ruakaka, New Zealand [Y]	CP137247 Nanopore/Illumina	B-anz5	21	**3–0**
CCCM 129	Chinook salmon (*Oncorhynchus tshawytscha*)	2021	Clay Point, New Zealand [Y]	CP137246 Nanopore/Illumina	B-anz1	**188**	2–1

(*continued on next page*)

Table 1 (*continued*)

Isolate ID	Host	Year	Origin	Genome accession, sequencing platform/s	Clade	MLST	O-AGC
CCCM 131	Chinook salmon (*Oncorhynchus tshawytscha*)	2021	Ōtānerau, New Zealand [Y]	CP137245 Nanopore/Illumina	B-anz5	**189**	**3–0**
CCCM 133	Chinook salmon (*Oncorhynchus tshawytscha*)	2021	Ōtānerau, New Zealand [Y]	CP137244 Nanopore/Illumina	B-anz1	**179**	2–1
CCCM 139	Chinook salmon (*Oncorhynchus tshawytscha*)	2021	Clay Point, New Zealand [Y]	CP137243 Nanopore/Illumina	B-anz1	**179**	2–1
CCCM 140	Chinook salmon (*Oncorhynchus tshawytscha*)	2021	Ngāmahau, New Zealand [Y]	CP137242 Nanopore/Illumina	B	**190**	2–1
CCCM 152	Chinook salmon (*Oncorhynchus tshawytscha*)	2021	Te Pangu, New Zealand [Y]	CP137241 Nanopore/Illumina	B	**191**	2–1

TCFB - Tasmanian Collection of Fish Bacteria (Tasmania, Australia); DPIF - Department of Primary Industry and Fisheries (Tasmania, Australia); CCCM - Cawthron Culture Collection of Microorganisms (Nelson, New Zealand); NCIMB - National Collection of Industrial, Food and Marine Bacteria (Aberdeen, Scotland); MLST - Multilocus Sequence Type; O-AGC – O-antigen gene cluster type. The notation '*' and '[Y]' indicate farms located at Pelorus Sound and Queen Charlotte Sound, respectively. Notation '[M 2–1]' indicates that the true O-AGC type 3–2 of the isolate TCFB 0809 was (mis)reported as type 2–1 in Lopez et all. Due to the wrong genome being originally deposited at PubMLST database. Letters in the 'Clade' column correspond to phylogenetic clades (Figs. 1, 2). inferred from non-recombinant core genome SNPs. Bold font indicates novel/potentially endemic MLST types, and potentially endemic (type 3–2) or rare (type 4–0, 3–0) O-ACG types.

and Exonuclease VII to remove failed ligation products, and purified with AMPure PB beads. The pooled samples were size selected using the BluePippin instrument (Sage Science) with a dye-free, 0.75% agarose cassette and S1 marker (Sage Science) and the 0.75% DF Marker S1 High-Pass 6 kb – 10 kb v3 run protocol, with a BPstart cut-off of 6000 bases. The final library was quantified using by Qubit fluorometer using dsDNA HS assay kit (Invitrogen), and fragment size assessed using the Perkin Elmer LabChip GX with the gDNA Reagent kit (Perkin Elmer).

Sequencing was performed using the PacBio Sequel system (software/chemistry v6.0.0). The pooled library was prepared for sequencing according to the SMRT Link sample setup calculator, following the standard protocol for diffusion loading with AMPure PB bead purification, using Sequencing Primer v4, Sequel Binding Kit v3.0 and the Sequel DNA Internal Control v3. The polymerase-bound library pool was sequenced on one SMRT Cell with a 10 h movie time plus a 2 h pre-extension using the Sequel Sequencing Kit 3.0 (PacBio, 101–597-900) and a Sequel SMRT Cell 1 M v3 (PacBio, 101–531-000). Library preparation and sequencing was performed at the Institute for Molecular Bioscience Sequencing Facility at The University of Queensland.

2.2.3. Nanopore MinION

Nanopore MinION sequencing was performed on gDNA extracted from all New Zealand isolates included in the study (CCCM isolates, $n = 31$), four Tasmanian isolates (TCFB 0648, 0022, 4540, 0813), and overseas strains NCIMB 2154^T and NCIMB 2158, at the University of Queensland (Table 1). gDNA was extracted using Quick DNA Miniprep Plus kit (ZymoResearch) and sequencing libraries were prepared with Rapid Barcoding Kit SQK-RBK004 (R9). This is with the exception of TCFB 0648 for which DNA was extracted using CTAB method (Wilson, 2001), and library prepared using Ligation Sequencing Kit SQK-LSK109 (R9) and barcoded using Native Barcoding Expansion Kit EXP-NBD104. Quantification of samples and sequencing libraries was performed with a Qubit fluorometer using dsDNA BR assay kit (Invitrogen). Barcoded libraries comprising 12 samples were sequenced on FLO-MIN106D flow cells (R9).

2.3. Genome assembly, annotation, and phylogenetic reconstruction

2.3.1. De novo assembly of Illumina reads

De novo draft assemblies were generated for isolates which were only sequenced on the Illumina platform (Table 1). Raw Illumina reads were examined in FastQC (*http://www.bioinformatics.babraham.ac.uk/projects/fastqc*). Sequences were quality trimmed with FastP v0.19.10 (Chen et al., 2018), which removed the *per*-base nucleotide content anomaly found in the first fifteen base pairs of Nextera XT libraries and any poly-G in the 3-prime end of the reads (low signal regions as a result of the two-colour base-calling protocol of the Nextseq500 system). After trimming, reads were assembled using Shovill pipeline v 1.1.0 (https://github.com/tseemann/shovill), which employs SPAdes assembler (Bankevich et al., 2012) with '–isolate', '-merged', ' –minlen 150', and '–mincov 4' flags. Quality of the assemblies was assessed with QUAST (Gurevich et al., 2013). Contigs were reordered using Mauve (Rissman et al., 2009), with respect to PacBio/Illumina complete genome of TCFB 4574 isolate (accession number CP137729) for draft assemblies of TCFB 4579 and TCFB 0814 genomes, and with respect to Nanopore/Illumina complete genome of TCFB 0648 isolate (CP137732) for all other draft assemblies. After submission to NCBI, contigs were processed by NCBI Foreign Contamination Screen tool.

2.3.2. PacBio-Illumina and MinION-Illumina hybrid assembly

Complete, high-accuracy genomes were generated using a hybrid approach (Sanderson et al., 2023), where both long and short reads were available (Table 1). Nanopore reads (or PacBio reads in the case of TCFB 4574 genome) were used to obtain closed genomes using Flye v 2.9 assembler (Kolmogorov et al., 2019), and assembly graphs were visualised in Bandage (Bioinformatics Application for Navigating *De novo* Assembly Graphs Easily) (Wick et al., 2015). Flye assemblies were error-corrected *via* mapping of the quality-trimmed Illumina reads using unicycler_polish command in Unicycler v 0.4.8 (Wick et al., 2017). Final error-corrected assemblies were re-oriented to start at bacterial origin of replication *dnaA* using fixstart command in Circlator (Hunt et al., 2015).

2.3.3. Genome annotation and browsing

Initial annotation of the complete and draft assemblies was performed with Prokka v 1. 12 (Seemann, 2014), and PGAP annotation was performed upon submission to NCBI Genome (Tatusova et al., 2016). Genome visualization and comparative analysis were carried out using Geneious Prime 2023.0.4 (https://www.geneious.com). The putative region encoding capsular polysaccharide biosynthesis genes (K-AGC) was initially located by presence of *wcaJ* gene in Prokka-annotated assemblies (SG507_09195 locus tag in PGAP-annotated assembly of the type strain (CP138495), encoding the glycosytransferase which initiates colanic acid synthesis (Pal et al., 2019). Operon and promoter predictions for surrounding gene annotations relevant to polysaccharide biosynthesis were carried out using SoftBerry tools FgenesB and BROM respectively (http://www.softberry.com). Multiple alignments of gene clusters were carried out using Clustal Omega 1.2.3 (Sievers and Higgins, 2018) plug-in.

2.3.4. Phylogenetic reconstruction

Core genome alignment was performed using Parsnp v 1.2 (Treangen et al., 2014) using UQ_NCIMB2154 genome assembly (CP138495) as reference. Extended multi-FASTA alignment was converted to multi-

FASTA using harvest-tools (https://github.com/marbl/harvest-tools) and areas of recombination were identified using Gubbins (Croucher et al., 2015). Alignment of recombination filtered (non-recombinant) core-genome variants was used to infer phylogenetic relationships among isolates using RAxML-NG and GTR + G + ASC_FELS(2,389,589) model (GTR + Gamma model with Felsenstein's ascertainment bias correction of the branch length accounting for 2,373,373 invariant core genome sites) and a bootstrap support of 1000 iterations (Kozlov et al., 2019; Leache et al., 2015). Phylogenetic trees were visualised and annotated in iTOL v6 (Letunic and Bork, 2021). Nodes with bootstrap support under 50 were collapsed. To identify whether O-AGC type 4–0 isolates USC SE30.1 and USC SP9.1 from Spain belong to C1 or C2 clade, their genome assemblies were retrieved from NCBI Genome (GCF_902705525.1, GCF_902705515.1) and phylogenetic relationships inferred from core genome SNP by approximately-maximum-likelihood criterion (FastTree2) (Price et al., 2010) using Parsnp v 1.2.

2.4. MLST typing

For TCFB isolates, trimmed Illumina Nextseq500 reads were used to identify MLST sequence type *in silico* using SRST2 (Inouye et al., 2014) based on 7 alleles (Habib et al., 2014). Novel MLST alleles and/or sequence types were confirmed by Sanger sequencing of PCR amplicons using published amplification and sequencing primers (Habib et al., 2014). PCR was performed with Q5® High-Fidelity 2× Master Mix (NEB) and 1 μM of primers which were annealed at 60°C for *atpA, dnaK, infB, rlmN, tgt* loci and at 55 °C for *glyA* locus (Habib et al., 2014), with 3 ng gDNA template in 15 μL reactions. PCR with Q5 polymerase was not achieved for *gyrB* locus (Habib et al., 2014), and instead was performed using OneTaq® 2× Master Mix with Standard Buffer (NEB), and 0.6 μM primers which were annealed at 55 °C. It may be useful to re-design the *gyrB* primer/s so that robust amplification can be achieved with high-fidelity PCR kits and/or relatively low template inputs. PCR products were enzymatically purified (shrimp alkaline phosphatase (NEB), exonuclease 1 (NEB)) and sequenced at the Australian Genome Research Facility (AGRF, Brisbane). For CCCM isolates, MLST profiles were derived by BLAST of the Nanopore-Illumina hybrid genome assemblies against PubMLST database (Jolley et al., 2018). All novel allele and/or sequence types were submitted to PubMLST database (Jolley et al., 2018) and identifiers assigned to them (Supplementary Table 3).

2.5. O-AGC typing

Molecular O-AGC types were initially inferred *in silico* by mapping of the published primers (Lopez et al., 2022) to genome assemblies. Identified O-AGC types were confirmed by O-AGC multiplex PCR amplification exactly as described (Lopez et al., 2022) for CCCM isolates in (Kumanan et al., 2024). For TCFB isolates, the described multiplex PCR was optimised for OneTaq® 2× Master Mix with Standard Buffer kit (NEB). For the latter amplification, P1, P4, and P6 reactions' primers were used at 0.2 μM concentration (recommended by the kit manufacturer), P5 reaction primers at 0.4 μM concentration, and P2/3 reaction primers at 1.6 μM concentration, and were annealed at 42 °C for 1 min.

2.6. Serological characterisation

Dot-blot assay was used for *T. maritimum* serotyping due to the difficulties in obtaining a homogenous suspension for macroscopic agglutination test (Avendaño-Herrera et al., 2004a). To prepare whole-cell killed antigens in the Tasmanian serotyping assay, bacterial cultures were grown to 10^9 cells/mL in MSB as described in Section 2.1, inactivated with 1% formalin, and processed by Dounce homogeniser to break up cell clumps. These antigen preparations were added at 100 μL/well to duplicate wells in a dot-blotting apparatus (Millipore, USA) fitted with nitrocellulose membrane (BioRad, Gladesville, NSW). The membrane was blocked with 3% *w/v* bovine serum albumin (BSA, Bovogen) in PBST (1× phosphate buffered saline with 0.05% *v*/v Tween® 20, Sigma-Aldrich) for 1 h at 18°C with agitation. Membranes were then probed with rabbit antisera raised against six *T. maritimum* isolates originally assigned by NRE Tasmania as serotype A (anti-TFCF 4635; anti-TCFB 4538; anti-TCFB 0482), serotype B (anti-TCFB 3289; anti-TCFB 0492) and serotype C (anti-TCFB 4574) (Table 2). Membranes were incubated overnight at 4°C and then agitated for 30 min at 18°C in antisera prepared in 1% w/v BSA in PBST with the following dilutions of antisera: 1:800 for anti-TCFB 0482, 1:400 for anti-TCFB 0492, 1:1600 for anti-TCFB 3289, 1:200 for anti-TCFB 4538, 1:200 for anti-TCFB 4574 and 1:6400 for anti-TCFB 4635. Membranes were washed three times with PBST and then probed with alkaline phosphatase-conjugated sheep anti-rabbit IgG (Chemicon) diluted 1:5000 in 1% w/v BSA in PBST, 1 h at 18°C, with agitation. Membranes were washed three times with PBST, once with TBST (1× Tris buffered saline, 0.05% v/v Tween® 20) and once with TBS (Tris buffered saline). The colour reaction was developed with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium solution (BCIP/NBT, Sigma Aldrich) with agitation. Membranes were done in triplicate for each six antisera and isolates judged positive when similar in colour to homologous control antigen (whole-cell antigen from isolates used to raise the antisera). A positive colour reaction across two or more membranes was considered an overall positive result for that antiserum. The serotyping performed in New Zealand was following the above Tasmanian method with minor modifications, and described in detail elsewhere (Kumanan et al., 2024). The assay was optimised and performed using three Tasmanian antisera against distinct NRE-assigned serotypes (anti-TCFB 4635 (serotype A); anti-TCFB 3289 (serotype B); and anti-TCFB 4574 (serotype C), and control preparations of their respective antigens from Tasmania.

3. Results and discussion

3.1. Genome assemblies

Despite significant tenacibaculosis research conducted by multiple research groups in the last 15 years, pathogenicity and serological diversity of *T. maritimum* remain poorly understood and effective vaccines for salmonids are lacking (Mabrok et al., 2022). Whole-genome comparative genomics has proved useful and robust in guided vaccine development and establishment of consistent molecular serotyping methods in a diverse array of fish pathogenic bacteria, including the salmonid pathogen *Yersinia ruckeri* (Barnes et al., 2016). Yet currently, genomic data is scarce for *T. maritimum,* with only a three complete genomes available to date at NCBI: NCIMB 2154^T type strain genome (LT634361.1) deposited in 2017, TM-KORJJ isolate genome (CP020822.1) deposited in 2019, and a very recently deposited genome of SP9.1 isolate (CP020822.1).

We produced genomic data for 72 isolates of *T. maritimum* from Oceania, collected in Tasmania between 1989 and 2018 (41 isolates; TCFB collection) and New Zealand in 2020–21 (31 isolates; CCCM collection), as well as three strains from overseas (Table 1). Complete high-quality genome assemblies were generated *via* long/short read hybrid approach for all CCCM isolates, five TCFB isolates, NCIMB 2154^T and NCIMB 2158 overseas strains (Table 1, Supplementary Table 1). For this, long reads from Nanopore sequencing platform (or PacBio platform in the case of TCFB 4574 isolate) were assembled into closed circular contigs, which in all cases was a single chromosome of 3.34–3.5 Mb genome size as expected for *T. maritimum* (Supplementary Table 1). To achieve high per-base accuracy, long-read assemblies were error-corrected ('polished') using short reads from the Illumina platform. Contig-level *de novo* genome assemblies were generated for the 37 TCFB isolates that were only sequenced on Illumina platform (Table 1; Suppl. Table 2).

Table 2
Dot-blot serotyping of *Tenacibaculum maritimum* isolates held at Tasmanian Collection of Fish Bacteria (TCFB).

Isolate	O-AGC type	Antisera to (isolate, O-AGC, clade)						Predicted serotype	Recorded serotype	Clade	MLST
		TCFB 3289, 2–1, B-anz3	TCFB 0492, 2–1, B-anz4	TCFB 0482, 3–0, B-anz5	TCFB 4635, 3–1, C1	TCFB 4538, 3–2, B-anz4	TCFB 4574, 4–0. C2				
NCIMB 2158	2–1	+	+	−	−	−	−	O2	O2	B	10
TCFB 0188	2–1	+	+	−	−	−	−	O2	O2	B-anz2	132
TCFB 4542	2–1	+	+	−	−	−	−	O2	O2	B-anz2	140
TCFB 0663	2–1	+	+	−	−	−	−	O2	O2	B-anz3	128
TCFB 0813	2–1	+	+	−	−	−	−	O2	O2	B-anz3	128
TCFB 0856	2–1	+	+	−	−	−	−	O2	O2	B-anz3	128
TCFB 0492	2–1	+	+	−	−	−	−	O2	O2	B-anz4	22
TCFB 0512	2–1	+	+	−	−	−	−	O2	O2	B-anz4	22
TCFB 0648	2–1	+	+	−	−	−	−	O2	O2	B-anz4	127
TCFB 0022	2–1	+	−	−	−	−	−	O2	O2*	B-anz3	126
TCFB 0811	2–1	+	+	−	−	+	−	O2	O2/O3*	B-anz2	135
TCFB 3289	2–1	+	+	−	−	−	+	O2	O2/O4	B-anz3	139
TCFB 4540	3–2	+	−	−	−	−	−	O3	**O2***	B-anz4	20
TCFB 4635	3–1	−	−	+	+	+	−	O3	O3	C1	142
TCFB 0024	3–2	−	−	+	+	+	−	O3	O3	C1	131
TCFB 0482	3–0	−	−	+	−	−	−	O3	O3*	B-anz5	21
TCFB 0509	3–0	−	−	+	−	−	−	O3	O3*	B-anz5	21
TCFB 0418	3–2	−	−	+	−	−	−	O3	O3*	B-anz4	19
TCFB 2947	3–2	−	−	+	−	−	−	O3	O3*	B-anz4	19
TCFB 3064	3–1	−	−	+	+	−	−	O3	O3*	B-anz4	129
TCFB 0639	3–2	−	−	+	+	−	−	O3	O3*	C1	133
TCFB 0759	3–2	−	−	+	+	−	−	O3	O3*	C1	134
TCFB 0853	3–2	−	−	+	+	−	−	O3	O3*	C1	134
TCFB 0809	3–2	−	−	−	+	−	−	O3	O3*	C1	134
TCFB 0518	3–2	−	−	−	+	−	−	O3	O3*	C1	24
TCFB 0019	3–2	+	+	+	−	+		O3	O2/O3*	B-anz4	19
TCFB 0023	3–2	+	+	+	−	+	−	O3	O2/O3*	B-anz4	19
TCFB 0187	3–2	+	+	+	−	+	−	O3	O2/O3*	B-anz4	19
TCFB 4536	3–2	+	+	+	−	+	−	O3	O2/O3*	B-anz4	20
TCFB 4646	3–2	+	+	+	−	+	−	O3	O2/O3*	B-anz4	130
TCFB 4538	3–2	+		+	−	+	−	O3	O2*/O3*	B-anz4	130
TCFB 0031	3–2	+		+	−	+	−	O3	O2*/O3*	B-anz4	19
TCFB 4607	3–2	+		+	−	+	−	O3	O2/O3*	B-anz4	19
TCFB 1813	3–2	+		+	−	−	−	O3	O2/O3*	B-anz4	20
TCFB 0543	3–0	+		+	+	+	−	O3	O3/O2*	B-anz5	21
NCIMB 2154 ^{T}	1–0	−	−	−	+	−	−	O1	**O3***	B	1
TCFB 4574	4–0	−	−	−	−	−	+	O4	O4	C2	141
TCFB 4579	4–0	−	−	−	−	−	+	O4	O4	C2	141
TCFB 1513	1–0	−	−	−	−	−	−	O1	(O1)	B	137
Baxa lyl-1	1–0	−	−	−	−	−	−	O1	(O1)	B	25
TCFB 2854	2–1	−	−	−	−	−	−	O2	(O1)	B-anz2	138
TCFB 0510	2–1	−	−	−	−	−	−	O2	(O1)	B-anz4	21
TCFB 0638	3–2	−	−	−	−	−	−	O3	(O1)	C1	133
TCFB 0814	4–0	−	−	−	−	−	−	O4	(O1)	C2	136

NCIMB - National Collection of Industrial, Food and Marine Bacteria; MLST - Multilocus Sequence Type; O-AGC – O-antigen gene cluster. Letters in the 'Clade' column correspond to phylogenetic clades in Fig. 1–2. Recorded serotypes notations: '*' – partial serotype assignment, negative for some of the same O-AGC type antisera; '/' – mixed serotype assignment positive for some/all antisera from different O-AGC type; (O1) – inferred serotype assignment to serotype O1, absence of reaction by O-AGC type 1 isolates to all antisera; in bold are assignments with complete lack of concordance to serotypes predicted by O-AGC typing.

3.2. Core genome phylogeny

Phylogeny of the 75 isolates used in the study was reconstructed by maximum likelihood (ML) inference from non-recombinant core genome single nucleotide polymorphism (SNPs) accounting for the number of invariant sites (Kozlov et al., 2019; Leache et al., 2015). Alignment of core genomes (Parsnp) identified 2,546,437 loci of which 156,848 were polymorphic. Of the latter, 88,314 SNPs were identified to be within areas of recombination by ML (Gubbins(Croucher et al., 2015)) and were removed from analysis. The remaining 68,534 non-recombinant core genome SNPs were used to reconstruct the phylogeny by ML (RAxML-NG) with Felsenstein's ascertainment bias correction of the branch length to account for 2,389,589 invariant core genome loci.

The majority of isolates within the dataset (n = 66) are closely related, indicated by the short branch lengths, and belong to the major phylogenetic lineage which includes all the New Zealand isolates (n = 31), most of the Tasmanian isolates (n = 30), and three strains from overseas (Fig. 1). The exception is within a group of 11 Tasmanian isolates which share a different common ancestor. These belong to a separate lineage splitting into two clades: 8-isolate clade found on a long branch and a 3-isolate clade on a very long branch (Fig. 1).

Several *T. maritimum* isolates from the major clade (NCIMB2154^{T} (Japan), NCIMB2158 (Scotland), and Tasmanian isolates TCFB 0031, 0492, 0510 collected in 1989–90) were used to build a global *T. maritimum* phylogenetic tree from SNPs within MLST loci (Habib et al., 2014). In the latter phylogeny, these isolates were found within a large cosmopolitan clade comprising isolates from various geographic regions, years, and hosts, which was named clade 'B'. Hence, we also assigned 'B'identifier to this diverse monophyletic group. Despite the overall close relatedness of isolates within lineage B, there was a detectable degree of endemism for both Tasmanian and New Zealand *T. maritimum* populations (Fig. 1–2). Sub-clade B-anz1 contained only New Zealand isolates (n = 4), and sub-clades B-anz2 and B-anz3 were

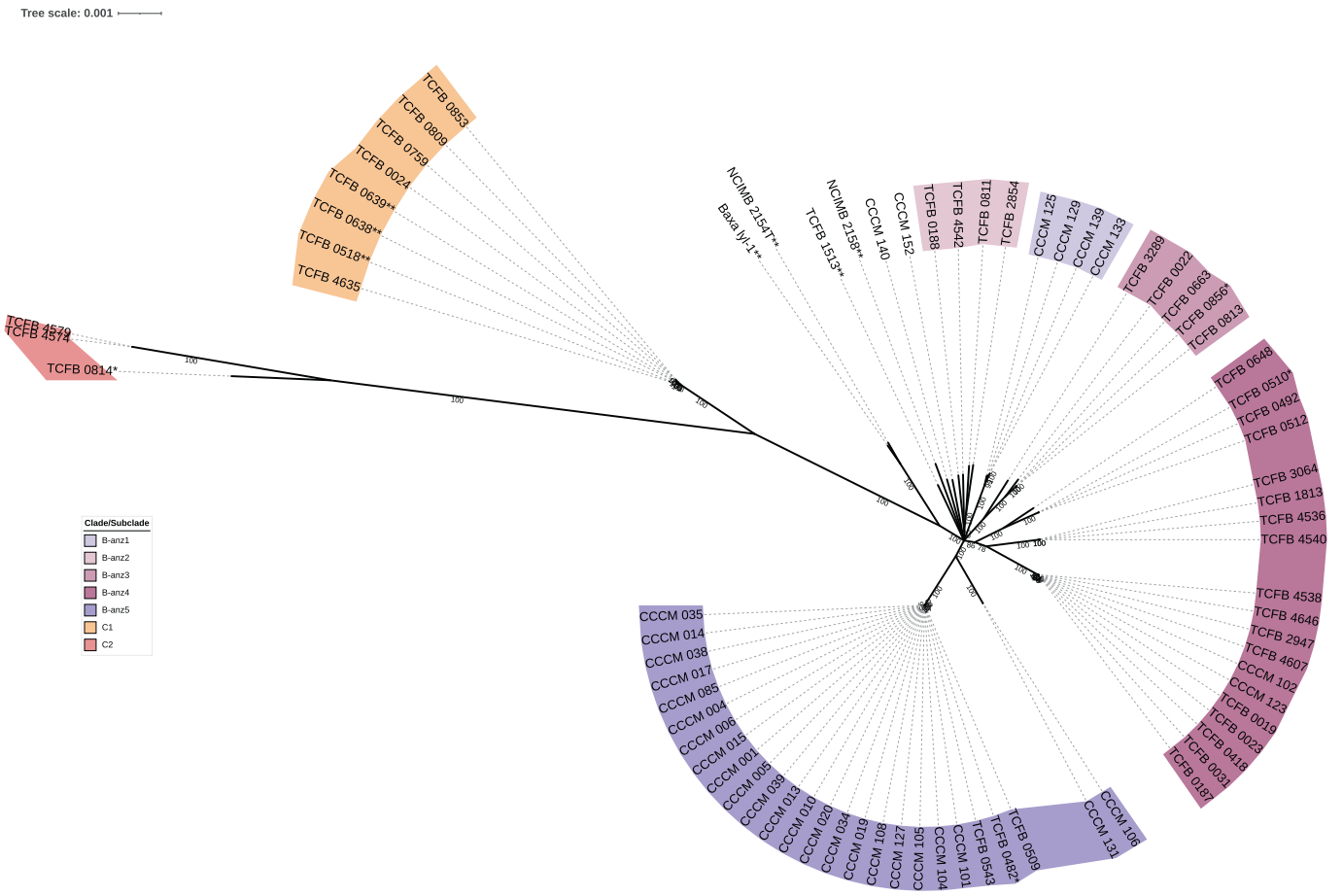

Fig. 1. Phylogenetic relationships among 75 isolates of *T. maritimum* inferred by Maximum Likelihood approach from 68,534 non-recombinant core genome SNPs, including Felsenstein's ascertainment bias correction for 2,389,589 invariant core genome loci. TCFB - Tasmanian Collection of Fish Bacteria (TCFB); CCCM - Cawthron Culture Collection of Microorganisms; NCIMB - National Collection of Industrial, Food and Marine Bacteria. '*' - isolates from trout, '**' – isolates from non-salmonid species. A – unrooted phylogram, B – unrooted cladogram.

comprised solely of Tasmanian isolates ($n = 4$ and $n = 5$, respectively). Sub-clade B-anz4 contained 17 Tasmanian isolates and only two New Zealand isolates, and, conversely, clade B-anz5 contained 23 New Zealand isolates and only three Tasmanian isolates. Interestingly, the latter three Tasmanian isolates (TCFB 0482, 0509, 0543) were isolated from Atlantic salmon in 1989 soon after the aquaculture industry was established in Tasmania, with no further isolations of this phylogenetic group in Tasmania thenceforth (Fig. 1–2, Table 1). The absence of more recent B-anz5 clade isolates from Tasmania, and current predominance of B-anz5 clade isolates in New Zealand, suggest that this evolutionary lineage was not successful in the Tasmanian environment but has become established within Chinook salmon aquaculture in New Zealand. Potentially, this could be due to the use of B-anz5 isolates in vaccines (unpublished information, NRE Tasmania). In contrast, subclades B-anz2, B-anz3, and B-anz4, contained both early and relatively recent Tasmanian isolates, which indicates these lineages have been successful in Tasmania and have persisted over the three decades covered by this study (Fig. 1–2, Table 1).

Persistence over time is also evident for the two minor clades ($n = 8$ and $n = 3$ isolates) comprising 11 evolutionary distant isolates from Tasmania (Fig. 1–2, Table 1). One of the isolates from 8-isolate clade, TCFB 0518, was used in MLST phylogeny where it was grouped into clade named as 'C' (Habib et al., 2014). Consequently, we named Tasmanian 8-isolate clade as 'C1' and 3-isolate clade as 'C2'. Such nomenclature ensures consistency with previous studies and shows that although these lineages share a common ancestor, they are nonetheless highly divergent groups, indicated by long phylogenetic branches (Fig. 1). In addition, different O-AGC types are found in clade C1 and C2: a seemingly endemic to Oceania type 3–2 prevails in clade C1, while isolates in C2 contain another rare O-AGC type 4–0 (Section 3.4.2, Fig. 2). The latter was found in Spanish isolates USC SE30.1 and USC SP9.1 which were grouped into 'C 'clade in MLST phylogeny (Habib et al., 2014; Lopez et al., 2022). Inclusion of this isolates into core genome phylogeny within our dataset has confirmed that they are phylogenetically distinct from clade C1 and belong to the C2 clade (Suppl. Fig. 1). Thus, group 'C' identified by MLST phylogeny comprises two distinct evolutionary lineages which were resolved by core genome phylogeny in this study, and named as 'C1' and 'C2'. For clade C2 there are two complete genomes available: a recently deposited assembly of Spanish isolate SP9.1 (CP020822.1, PacBio) and assembly of the Tasmanian isolate TCFB 4574 (CP137729; PacBio/Illumina) from this study (Table 1, Suppl. Table 1). Notably, there could be some significant differences between these Spanish and Tasmanian isolations as they are found in different subclades within C2 lineage (Suppl. Fig. 1). The clade C1 has no representative strains from overseas to date, and thus, it appears that eight contig-level assemblies generated by this study are the best currently available representative genomes (Table 1, Suppl. Table 2).

Interestingly, there were no representatives of clade 'A', a third phylogenetic clade resolved by MLST phylogeny (Habib et al., 2014) in our dataset. Among other potential factors, this could be linked to O-AGC type 1–0 found in this clade (see Section 3.4).

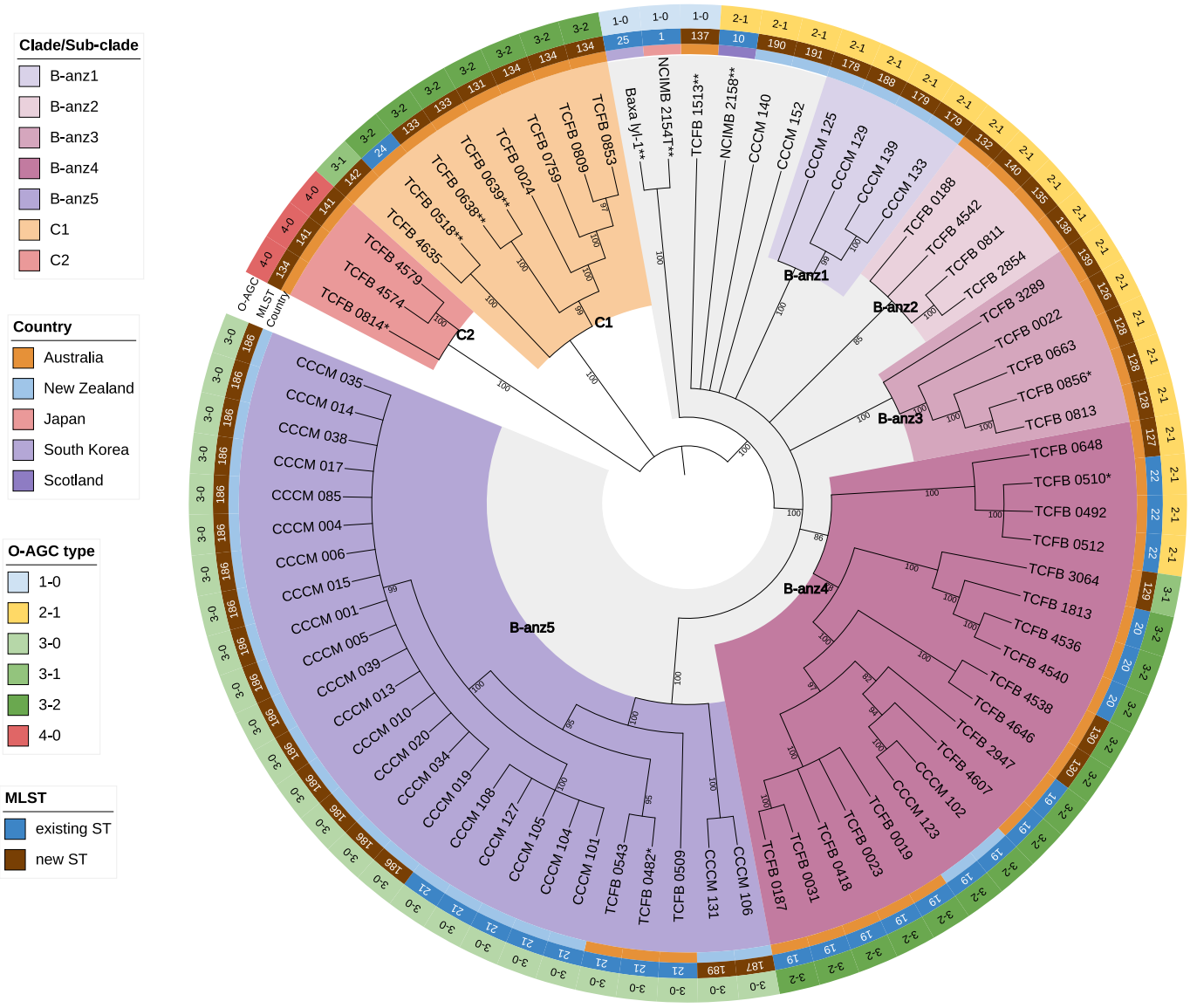

Fig. 2. Midpoint rooted cladogram annotated with country of isolation, Multilocus Sequence Type (MLST), and O-antigen gene cluster (O-AGC) type. TCFB - Tasmanian Collection of Fish Bacteria (TCFB); CCCM - Cawthron Culture Collection of Microorganisms; NCIMB - National Collection of Industrial, Food and Marine Bacteria; ST - sequence type. '*' - isolates from trout, '**' – isolates from non-salmonid species.

3.3. Multilocus sequence typing

The MLST scheme based on 7 loci (*atpA, dnaK, glyA, gyrB, infB, rlmN, tgt*) (Habib et al., 2014) was applied to the isolates in the present study. With minor exceptions, sub-clades within major phylogenetic lineages had similar but distinct sequence types (ST) demonstrating robustness and high resolution of the MLST scheme (Fig. 2). Out of 72 *T. maritimum* isolates from Tasmania and New Zealand, 48 isolates had new ST, indicative of localized evolution of *T. maritimum* in the Tasman sea/Oceania (Fig. 2; Suppl. Table 3). Out of 48 isolates with new ST, 24 were from Tasmania and 24 from New Zealand, and, importantly, both novel and existing ST differed between the two regions, consistent with independent evolution of *T. maritimum* in each location. Among 41 isolates from Tasmania, 24 isolates belonged to one of the 17 new ST, which comprised 12 novel ST with new alleles (assigned ST – 131-142) and five novel ST with new combinations of existing alleles (assigned ST – 126-130). Among 31 isolates from New Zealand, 24 isolates belonged to one of the six new ST, which were new combinations of known alleles (assigned ST – 186-189, 191) (Fig. 2, Suppl. Table 3).

Most sub-clades appear to be highly endemic, with some (B-anz1–3, C2) containing solely new ST and some (B-anz5, C1) primarily new STs and few existing, likely ancestral, STs. Although clade B-anz4 comprised of mostly previously assigned STs, relatively recent isolates (TCFB 3064, 4538, 4646, 0648) have new STs (Fig. 2), and, except for four isolates from 1989 and one isolate from 2013, all isolates in this lineage had potentially endemic O-antigen type 3–2 (see below).

3.4. O-AGC typing

3.4.1. Distribution of O-AGC types

In all cases, mPCR products were consistent with *in silico* identified O-AGC types (Suppl. Fig. 2). Somewhat unexpectedly for genetic determinants of a variable antigen, O-AGC types correlated strongly with phylogenetic affiliation and overall, were highly conserved within phylogenetic clades or/and subclades (Fig. 2, Table 1). Distribution of O-AGC types differed between TCFB and CCCM isolates, consistent with that seen with MLST typing, distinguishing the evolution of *T. maritimum* in Tasmania and New Zealand (Fig. 2). The majority of Tasmanian isolates (46%) were a unique O-AGC type 3–2, which to date has only been found in Australia and New Zealand (Kumanan et al., 2024; Lopez et al.,

2022). A total of 31% of the Tasmanian isolates shared the globally common O-AGC type 2–1, while only a handful of isolates were of the globally common types 3–1 (5%) and 1–0 (2%), and the globally rare types 3–0 (7%) and 4–0 (7%) (Lopez et al., 2022). In the New Zealand dataset, there were a few isolates (6%) belonging O-AGC type 3–2 potentially endemic to Tasman sea/Oceania, 19% were from the globally common type O-AGC type 2–1, and the remaining majority were from the globally rare O-AGC type 3–0 (Kumanan et al., 2024; Lopez et al., 2022).

Interestingly, although *T. maritimum* appears to encounter distinct selective pressures in Tasmania and New Zealand, leading to separate distributions of O-AGC types, there is an indication of linked evolutionary history between these locations: a few Tasmanian isolates of AGC type 3–0 ($n = 3$) are found in clade B-anz5 pre-dominating in New Zealand, and inversely, a few New Zealand isolates of AGC type 3–2 ($n = 2$) are found withing clade B-anz4 pre-dominating in Tasmania.

3.4.2. O-AGC evolution

O-AGC types correlated strongly with phylogenetic position and there was a link between globally common O-AGC types and endemic MLST, and *vice versa*, in the clades and/or subclades. (Fig. 2, Table 1). For example, in clades B-anz1–3, a cosmopolitan O-AGC type 2–1 is found in a combination with new potentially endemic STs shared between isolates 1989 to 2018, suggesting that associated O-antigen variant was retained over time despite prolonged localized evolution. Conversely, isolates from clade B-anz4 with the existing globally common ST 19 and 20, have O-AGC type 3–2 potentially endemic to Tasman sea/Oceania, which in this instance has emerged without major alterations to the core genome. Importantly, the endemic O-AGC type 3–2, has also evolved independently in phylogenetically distant lineage C1 (Fig. 1–2), and in this case, it was associated with an endemic ST. Therefore, O-AGC type 3–2 is almost certainly established in Tasmanian aquaculture, indicated by high prevalence, two independent emergence events, and persistence through time. Considering Tasmanian isolates of both O-AGC type 2–1 and 3–2 have been circulating in the environment since 1989, there is a strong indication that co-existence of these O-AGC types is favoured in local *T. maritimum* populations.

In contrast to Tasmania, where tenacibaculosis has been recognised as a threat in salmonid aquaculture for 30 years, there is a paucity of historic *T. maritimum* isolates in New Zealand. Nonetheless, a distribution of O-AGC types among recent New Zealand isolates suggests that co-existence of two distinct O-antigen variants is favoured. Consistent with that seen for Tasmania, O-AGC type 2–1 is common but less abundant in New Zealand isolates (Fig. 2; clade B-anz1). However, the predominant O-AGC type in New Zealand is type 3–0 (Fig. 2; clade B-anz5), not O-AGC type 3–2 as seen in Tasmania. According to Lopez et al. (2022), molecular subtypes within each major O-AGC type are expected to represent the same serotype, *i.e.*, O-AGC types 3–0 and 3–2 may be serologically identical. However, this may not be true for all the host species, and selective factors, other than the host's immune pressure may determine fitness of the O-antigen subtypes within local environments. For Tasmanian aquaculture, selection against O-AGC type 3–0 is supported by the historic presence (Fig. 2, Table 1; three TCFB isolates in clade B-anz5 collected in 1989) but no instances of its isolation in the last 30 years. For New Zealand, however, selection against 3–2 type is only suggested by its paucity among recent isolations; potentially, it may be an emerging variant which will increase over time.

Regarding other O-AGC types detected in Tasmania, a rare type 4–0 was found in phylogenetically distant clade C2, comprising one isolate from rainbow trout collected in 1999, and two isolates from Atlantic salmon collected in 2017 (Fig. 2, Table 1). A common type 3–1 was present in two isolates recovered from Atlantic salmon in 2013 and 2018; which were found among type 3–2 isolates in clades B and B-anz4, but had unique MLST profiles. Analysis of isolates from more recent tenacibaculosis outbreaks is required to reveal whether O-AGC types 3–1 or/and 4–0 have become established in Tasmania and represent a threat to salmonid aquaculture industry. Lastly, there was a single isolate with globally common O-AGC type 1–0, TFCB 1513. This isolate was recovered from striped trumpeter larvae housed in a recirculating aquaculture system facility in 2003, and did not group into any of the Tasmanian/New Zealand clades, occupying an individual phylogenetic branch within clade B. With the current evidence, it appears that the corresponding serotype O1 does not pose a threat to salmonid industries, at least in Oceania (Section 3.5).

3.5. Fish host specialisation

Regarding host species, isolates from rainbow trout (TCFB 0856, 0510, 0482, 0418) clustered tightly with Atlantic salmon isolates in all three clades (B, C1, C2) indicating a complete lack of *T. maritimum* host specificity within salmonids (Fig. 1–2). The lack of strict fish host specificity in general was supported by the grouping of isolates from salmonid and non-salmonid fish within clades B and C1. With a single exception (TCFB 1513, O-AGC type 1–0), isolates in these clades are of O-AGC types 2 and 3 expected to represent serotype O2 and O3, respectively, which were isolated from various fish taxa globally (Escribano et al., 2023; Lopez et al., 2022). Likewise, O-AGC type 4–0/serotype O4 found in clade C2 salmonids was previously detected in turbot and sole (Escribano et al., 2023). On the other hand, group 'A' resolved by MLST phylogeny comprised solely of South European non-salmonid isolates, mostly from *Sparidae* (Habib et al., 2014), and there were no representatives of this clade in our salmonid-biased dataset, despite sampling in Tasmania for over three decades (Table 1). Notably, group A isolates, show O-AGC type 1–0/serotype O1, which to date was only isolated from non-salmonid fish species (Escribano et al., 2023; Lopez et al., 2022), suggesting salmonids may not be susceptible, or be poorly susceptible to it. At the least, there is currently no indication that salmon industries in Oceania could benefit from protection against *T. maritimum* serotype O1.

3.6. (Dis)concordance between O-AGC typing and antiserum serotyping

3.6.1. Dot-blot serotyping of Tasmanian isolates; serological heterogeneity within O-AGC type 3–2

The expectation for the recently proposed O-antigen molecular typing scheme (Lopez et al., 2022) is that four major types of O-AGC (regardless of the subtype) correspond to serotypes O1, O2, O3, and O4 identified by conventional serotyping (Fernández-Álvarez et al., 2018; Mabrok et al., 2022). In general, this was true for serotyping of TCFB isolates using a dot-blot assay employing six antisera: anti-TCFB 4574 against O-AGC type 4–0, anti-TCFB 3289 and anti-TCFB 0492 against O-AGC type 2–1, and three antisera against O-AGC type 3 but of different subtypes (anti-TCFB 0482 against 3–0, anti-TCFB 4635 against 3–1, anti-TCFB 4538 against 3–2) (Table 2).

The most serologically distinct was O-AGC type 4–0, previously reported in Spanish isolates from Coho salmon (*Oncorhynchus kisutch*) assigned as serotype O4 (Lopez et al., 2022). There were only three isolates of this type, which comprised a phylogenetically distant clade C2 (Fig. 1). Indeed, anti-TCFB 4574 bound two isolates of the O-AGC 4–0 type collected from salmon in 2017 (itself, and TCFB 4579), and did not cross-react with isolates of other O-AGC types, with one exception (Table 2). In turn, the other five antisera against isolates from other O-AGC types did not react with O-AGC type 4–0 isolates. However, the anti-TCFB 4574 serum failed to recognize a third isolate of O-AGC type 4–0, TCFB 0814, which was isolated from rainbow trout in 1999 and has different MLST.

There were no antisera against O-AGC type 1–0 isolates used in serotyping the TCFB isolates, but all six antisera (against isolates with other O-AGC types) did not bind to the two isolates with O-AGC type 1–0: TCFB 1513 (Tasmania) and Baxa-lyl1 (Korea). Indeed, this is supportive of the latter isolates being assigned to a distinct serotype O1. The remaining O-AGC type 1–0 representative, type strain NCIMB 2154^{T}),

was positive with TCFB 4635 antiserum raised against O-AGC type 3–1, but traditional serotyping results for this isolate have been inconsistent between laboratories (Avendaño-Herrera et al., 2004a; Fernández-Álvarez and Santos, 2018).

O-AGC type 2–1 isolates were positive for both antisera against O-AGC type 2–1 (anti-TCFB 3289 and anti-TCFB 0492), with some exceptions; and negative with antisera against other O-AGC types, regardless of phylogenetic affiliation (Table 2, Fig. 2). This supports that O-AGC type 2–1 corresponds to a distinct serotype, which was assigned as O2 (Lopez et al., 2022).

There was much less correlation between the predicted and recorded serotypes for O-AGC type 3 isolates. Indeed, there was no significant binding of antisera against O-AGC type 3 (anti-TCFB 0482, anti-TCFB 4536, anti-TCFB 4638) to isolates from other O-AGC types (Table 2). However, only two O-AGC type 3 isolates were recognised by all three antisera against O-AGC type 3, and not recognised by any of the antisera from other O-AGC types, and thus could be fully assigned to serotype O3. In contrast, most of the O-AGC type 3 isolates were either not bound by one or two antisera against O-AGC type 3, bound by one or both antisera against O-AGC type 2, or both instances, leading to partial, mixed, or partial/mixed result of the serotype (Table 2).

Importantly, clear differences in dot-blot results between three antisera against O-AGC type 3 were strongly associated with phylogenetic position of isolates used for antisera production. Aside from a few exceptions, antiserum to TCFB 0482, from clade B-anz5, bound O-AGC type 3 isolates regardless of their phylogenetic affiliation, *i.e.* isolates from clades B-anz5, B-anz4, and C1, were recognised. Whereas antiserum to TCFB 4635, from clade C1, was only bound clade C1 isolates, and antiserum to TCFB 4538, from clade B-anz4, was only bound to isolates also in clade B-anz4. Moreover, the (mis)recognition of O-AGC type 3 isolates by O-AGC type 2 antisera was also strongly affected by phylogenetic affiliation. Antiserum to 2–1 isolates, TCFB 3289 from clade B-anz3 and TCFB 0492 from B-anz4, cross-reacted with many isolates of O-AGC type 3–2 from clade B-anz4 and almost none from clades B-anz45 and C1 (Table 2).

These results show relatively good correlation between O-antigen molecular typing and conventional serotyping for O-AGC types 1, 2, and 4. However, only a weak correlation is seen for O-AGC type 3 for which (mis)assignments to serotype/s were very common and strongly linked to phylogenetic position of the isolates. Considering high fitness of seemingly endemic O-antigen type 3–2 in Tasmania and decades of localized isolate evolution, it is conceivable that serologically distinct sub-types within type 3–2 have emerged. Although no difference was found in O-AGC gene content between O-AGC type 3–2 isolates from different phylogenetic groups, some clade-specific non-synonymous substitutions were found (data not shown). The antisera recognition results *per se* suggest that vaccine prepared from O-AGC type 3 isolate belonging to clade B-anz5 may be protective against O-AGC type 3 isolates from any clade. However, considering that Tasmanian isolates from clade B-anz5 have been last isolated in 1989, and accounting for the differences between piscine and mammalian immune responses, formulating a vaccine to containing two more recent O-AGC type 3–2 isolates, one from clade B-anz4 and one from clade C1 appears to be a better strategy.

3.6.2. *Dot-blot serotyping of New Zealand isolates; K-antigen gene cluster*

In contrast to serotyping of Tasmanian isolates, the dot-blot assay results for New Zealand isolates showed no correlation with the proposed O-AGC scheme as they could not distinguish between O-AGC types (Kumanan et al., 2024). Three distinct Tasmanian antisera were used for serotyping of CCCM isolates - anti-TCFB 4635, anti-TCFB 3289, and anti-TCFB 4574, against O-AGC types 3–1, 2–1, and 4–0 respectively. Most CCCM isolates were bound by all three cross-absorbed antisera regardless of their O-AGC type suggesting a conserved common antigen, which must have also been present in all three TCFB isolates used to prepare antisera. Yet, considering there was no major binding of the heterologous O-AGC types in the Tasmanian dot-blot assay, the common antigen was apparently much less abundant in Tasmanian cultures/whole-cell antigen preparations compared to those used in New Zealand.

Unlike when a purified single antigen (*e.g.* extracted LPS) is used for antisera serotyping, a whole-cell antisera-based serotyping heavily relies on the combined immunogenicity of all surface-exposed antigens. In gram-negative bacteria these may include surface polysaccharides (O- and K- antigens) and antigenic protein structures protruding through the polysaccharide layer such as flagellae, fimbriae, or pilli. The latter protein antigens are lacking in *T. maritimum* (Avendaño-Herrera et al., 2006), and although there can be some common cell-wall associated surface proteins present, these are typically masked from immune recognition by surface polysaccharides, O- or/and K-antigens (Cress et al., 2014; Dominguez-Medina et al., 2020; Zangari et al., 2021), and unlikely to compromise the O-antigen typing. Hence, capsular polysaccharide (exopolysaccharide, K- antigen) is the most likely common antigen which was bound by all three antisera in NZ assay resulting in lack of resolution of O-antigen type (Kumanan et al., 2024).

Capsular material was previously detected in *T. maritimum* isolates by electron microscopy regardless of their serotype (Avendaño-Herrera et al., 2006), yet the K-antigen encoding gene cluster (K-AGC) has not been described to date. The 'exopolysaccharide' cluster (locus tag: MARIT 2522–2537) predicted in the first complete *T. maritimum* genome of the type strain (Perez-Pascual et al., 2017) was subsequently identified as O-AGC (Lopez et al., 2022). Here we report for the first time a K-antigen biosynthesis gene cluster (K-AGC) of 16 kB located between 2,051,491 and 2,067,473 positions in UQ-NCIMB2154 genome assembly of the type strain (CP138495). It comprises 14 genes (locus tag: SG507_09190–09255) predicted to be transcribed from the reverse strand in two units (Fig. 3). Gene annotations for GDP-mannose 4,6-dehydratase (*gmd;* SG507_09245) and GDP-L-fucose synthase (*fcl;* SG507_09240) (Fig. 3), indicate that *T. maritimum* produces a colanic acid (CA) capsule or similar exopolysaccharide (Andrianopoulos et al., 1998; Stevenson et al., 1996). CA is produced by, and well-studied in, *Escherichia coli, S. enterica*, and *Klebsiella pneumoniae*, and known to be critical for biofilm formation (Pal et al., 2019; Pando et al., 2017; Sung et al., 2006). Indeed, *T. maritimum* is noted for its ability to aggregate and form biofilm structures, a characteristic that is reflected in the genus name *Tenacibaculum* (Levipan et al., 2019; Suzuki et al., 2001). It is also well-established that CA expression is up-regulated in response to environmental stress (Ebel and Trempy, 1999; Pando et al., 2017), and binding sites for stress-associated transcription factors are abundant within promoter regions predicted for putative K-AGC transcription units (Fig. 3). Unlike other types of capsular material (K-antigen) that are tightly associated with the cell surface, CA, also known as M-antigen, is a loose form of exopolysaccharide forming a mesh coat within biofilms. Importantly, it was found that when CA is overproduced it can covalently bind to the LPS core in the place of O-antigen, forming M_{LPS} (Meredith et al., 2007).

We propose that the lack of resolution of O-antigen type in the New Zealand dot-blot assay (i. e. positive reactions with antisera against heterologous O-antigen types), is most likely explained by higher amount of CA in NZ cultures. Although the same whole-cell antigen preparation protocol was followed by Tasmanian and New Zealand serotyping laboratories, it is conceivable that some minor differences in culture conditions have enhanced CA production and M_{LPS} formation in New Zealand cultures/antigen preparations. For instance, pH of the MSM broth was routinely adjusted to pH 7.4 in the case of TCFB cultures, and to pH 7.8 in the case of CCCM cultures. It appears that the latter were also grown with somewhat lower agitation compared to TCFB cultures. Alternatively, New Zealand isolates could produce more CA due to genetic factors, such as presence/absence of stress response regulators or transcription factors, mutations in these proteins or their targets, or presence of prophages (Kim et al., 2015; Zhang et al., 2008).

The discovered O-AGC cluster is highly conserved among New

locus_tag	Length	product
SG507_09255	1,680	hypothetical protein
SG507_09250	1,041	hypothetical protein
SG507_09245	1,119	GDP-mannose 4,6-dehydratase
SG507_09240	918	GDP-L-fucose synthase
SG507_09235	1,161	DegT/DnrJ/EryC1/StrS family aminotransferase
SG507_09230	1,251	oligosaccharide flippase family protein
SG507_09225	798	glycosyltransferase family 2 protein
SG507_09220	948	class I SAM-dependent methyltransferase
SG507_09215	1,233	O-antigen ligase family protein
SG507_09210	1,065	glycosyltransferase family 4 protein
SG507_09205	1,347	O-antigen ligase family protein
SG507_09200	1,119	glycosyltransferase
SG507_09195	1,356	exopolysaccharide biosynthesis polyprenyl glycosylphosphotransferase
SG507_09190	762	glycosyltransferase

> Operon 1 promoter

Length of sequence- 100
Threshold for promoters - 0.20
Number of predicted promoters - 1
Promoter Pos: 81 LDF- 5.81
-10 box at pos. 66 CGTTAATTT Score 48
-35 box at pos. 46 TTGGCT Score 33

Oligonucleotides from known TF binding sites:

For promoter at 81:
ihf: AAAGTATA at position 30 Score - 14
rpoD15: CTAAATAA at position 50 Score - 12
ihf: TTAATTTA at position 68 Score - 12
cpxR: TAAAACAA at position 90 Score - 9

> Operon 2 promoter

Length of sequence- 200
Threshold for promoters - 0.20
Number of predicted promoters - 1
Promoter Pos: 106 LDF- 4.76
-10 box at pos. 91 ACTTTTAAT Score 36
-35 box at pos. 69 TTGAAC Score 38

Oligonucleotides from known TF binding sites:

For promoter at 106:
soxS: AAACCATA at position 61 Score - 7
rpoD17: AATAAATA at position 97 Score - 11
lexA: ATAAATAA at position 98 Score - 14
rpoH3: AATAACTC at position 101 Score - 10

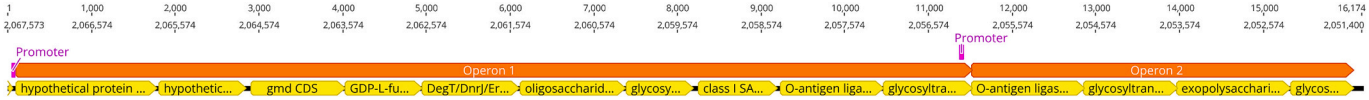

Fig. 3. Putative exopolysaccharide gene cluster (K-AGC) of *T. maritimum.* Region was extracted from genome assembly UQ_NCIMB2154 (CP138495) of the type strain. Gene annotations were performed with PGAP, operon and promoter annotations were added manually according to FGENESB and BROM (SoftBerry) predictions respectively. LDF - linear discriminant function, TF – transcription factor.

Zealand isolates. In all CCCM genomes ($n = 31$) it is nearly identical to the cluster in NCIMB2154^T (> 99.5% pairwise identity in multiple nucleotide alignment). The same is true for TCFB 4536 isolate (O-AGC type 3–1, clade C1), used to produce one of the typing antisera. The remaining two isolates used to produce the typing antisera, TCFB 3289 (O-AGC type 2–1, clade B-anz3) and TCFB 4574 (O-AGC type 4–0, clade C2) contain somewhat different versions of this cluster, with some variation in the middle of the locus (Suppl. Fig. 3). Tentatively, we name the above two versions of the O-AGC cluster as type 1–0 represented by NCIMB2154^T (CP138495; SG507_09190–09255) and type 1–1 represented by TCFB 4574 (CP137729; R8G65_09230–09285).

A separate study is required to characterise the diversity of *T. maritimum* K-AGC, and, most importantly, the effect of culture conditions on its expression, as well as expression *in vivo*. With respect to the latter, CA knockout mutant of *Salmonella* induced higher antibody titres in mice (Wang et al., 2013), so the opposite may be expected for isolates that overproduce *CA*. In contrast, A CA knockout mutant of *Klebsiella pneumoniae* appeared to be less immunogenic (Pal et al., 2019). Whichever applies to *T. maritimum*, it is likely that CA production is critical not only for achieving consistent serotyping with this pathogen, but also the immunogenicity/efficacy of the whole-cell vaccines against it.

4. Conclusions

The substantial amount of genomic sequence data from *T. maritimum,* including multiple high-quality complete closed genomes, generated here will assist investigation of disease etiology, and vaccine development and susbsequent stewardship for salmon aquaculture in Australia and New Zealand. Phylogenetic analysis coupled to MLST, O-AGC typing, and conventional antisera serotyping, indicate a high degree of geographic endemism in both Tasmania and New Zealand, and a need for autogenous whole-cell vaccines tailored for each location. There was only a partial concordance between O-AGC typing and molecular serotyping for Tasmanian isolates, importantly different serotypes were recorded for isolates with predominant, likely endemic, O-AGC type 3–2 belonging to different phylogenetic clades. In the case of New Zealand, there was no concordance as different O-AGC types were not serologically distinct in the dot blot assay. Overall, a vaccine comprising O-AGC type 2–1 and 3–0 isolates may be suitable for New Zealand farms at present. For Tasmania, a vaccine comprising O-AGC type 2–1, type 3–2 isolate from clade B-anz4, type 3–2 isolate from clade C1, and possibly also type 4–0 may be suitable.

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CRediT authorship contribution statement

O. Rudenko: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Data curation, Conceptualization. **C. Angelucci:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **K. Kumanan:** Writing – review & editing, Software, Methodology, Investigation. **L. Delisle:** Writing – review & editing, Methodology, Investigation. **J. Carson:** Writing – review & editing, Supervision, Resources. **R.N. Morrison:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Data curation. **K.S. Hutson:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **A.C. Barnes:** Writing – review & editing, Supervision, Software, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

Genome assemblies were deposited in Genome database at NCBI under BioProject PRJNA1031525.

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