RESEARCH PAPER



Comprehensive Genome Analysis of Planococcus sp. S3-L1 Isolated from Horseshoe Island in Antarctica Reveals Its **Biotechnological Potential**

Hilal Ay^{1,*}, Sibel Melisa Sahin², Muhammed Duman³, Nihed Ajmi⁴, Izzet Burcin Saticioglu³

¹Yildiz Technical University, Faculty of Arts and Science, Department of Molecular Biology and Genetics, Istanbul/Türkiye (34220). ²Ondokuz Mayis University, Institute of Graduate Studies, Samsun/Türkiye (55200).

³Bursa Uludag University, Faculty of Veterinary Medicine, Department of Aquatic Animal Diseases, Bursa/Türkiye (16059).

⁴Bursa Uludag University, Graduate School of Health Sciences, Bursa/Türkiye (16059).

Abstract

compounds.

How to Cite

Ay, H., Sahin, S.M., Duman, M., Ajmi, N., Saticioglu, I.B.(2025). Comprehensive Genome Analysis of Planococcus sp. S3-L1 Isolated from Horseshoe Island in Antarctica Reveals Its Biotechnological Potential. Turkish Journal of Fisheries and Aquatic Sciences, 25(6), TRJFAS27193. https://doi.org/10.4194/TRJFAS27193

This study explores the biotechnological potential of Planococcus strain S3-L1, isolated

from Horseshoe Island in Antarctica during the 6th Turkish Antarctic Expedition in 2022. Marine bacteria inhabiting polar environments play essential roles in ecosystem dynamics and biogeochemical cycles, largely due to their unique adaptations. The

Planococcus genus, noted for its halotolerance and ability to thrive in cold

environments, has garnered interest for its capability to produce bioactive metabolites, support bioremediation, and facilitate various industrial processes. In this research, Planococcus sp. S3-L1 was isolated and underwent 16S rRNA and whole-

genome analysis, revealing a close genetic affinity with Planococcus kocurii and

Planococcus faecalis. Genomic analysis identified genes responsible for carotenoid

pigment production and aromatic compound degradation, underscoring its potential

in environmental remediation and low-temperature pigment production.

Furthermore, quorum-quenching enzymes suggest that S3-L1 could serve as an

antimicrobial agent. These findings show that Planococcus sp. S3-L1 is a promising candidate for biotechnological applications, particularly within polar marine environments, where its cold adaptation and metabolic diversity offer potential benefits in aquaculture, bioremediation, and the synthesis of commercially valuable

Article History

Received 11 November 2024 Accepted 23 January 2025 First Online 07 February 2025

Corresponding Author

E-mail: hilal.ay@yildiz.edu.tr

Keywords Biotechnology Genomic analysis

Antarctic marine bacteria Horseshoe island Planococcus

Introduction

Microbial diversity in Antarctic marine waters is essential for maintaining ecosystem functions, driving biogeochemical cycles, and supporting the food web. The unique and dynamic conditions of the Southern Ocean foster a diverse range of microbial communities crucial for carbon sequestration and nutrient cycling. Understanding these microbial communities and their responses to environmental changes is vital for predicting the impacts of climate change on Antarctic marine ecosystems (Cavicchioli, 2015; Tonelli et al., 2021).

Microbes in Antarctic waters are key drivers of biogeochemical cycles, including the carbon, sulphur, and nitrogen cycles. They transform complex carbon compounds into simpler, bioavailable forms, facilitating ecosystem nutrient cycling and energy flow. Microbial community composition and diversity vary with depth and environmental gradients, with different taxa dominating at various depths and conditions. This diversity allows microbes to adapt to and thrive in the dynamic and extreme conditions of the Antarctic marine environment (Dutta et al., 2023; Murray & Grzymski, 2007; Signori et al., 2014). However, climate change is expected to impact the microbial communities in the

Antarctic marine environment significantly. Changes in temperature, ice cover, and nutrient availability will likely alter the composition and function of these communities, with potential consequences for the broader polar ecosystem (Castillo et al., 2022; Grattepanche et al., 2022).

Planococcus is a Gram-positive, halotolerant bacterial genus in the phylum Bacillota, commonly found in various habitats in Antarctica (See-Too et al., 2017). Planococcus species are predominantly found in cold environments, such as the Arctic permafrost, accounting for approximately 5.8% of the bacterial community (Lay et al., 2012). Planococcus species in Antarctica exhibit unique adaptations to extreme environmental conditions, making them valuable for various biotechnological applications due to their ability to produce valuable metabolites like carotenoids, exhibit quorum quenching activity, function enzymatically at low temperatures, and resist heavy metals. These traits make them promising candidates for applications in antioxidant production, bioremediation, and industrial processes requiring coldactive enzymes. The discovery of new species further underscores the potential for uncovering additional biotechnological uses (Kim et al., 2015; Margolles et al., 2012; See-Too et al., 2017; Styczynski et al., 2020). At that time, 30 type strains of Planococcus had been characterised, with species such as Planococcus antarcticus and Planococcus psychrophilus, isolated from cyanobacterial mats in Antarctic ponds, representing the primary species adapted to extreme environments (Reddy et al., 2002).

In this study, we aim to identify and characterize the genomic features of a *Planococcus* strain isolated from a water sample collected from the coast of the Horseshoe Island in Antarctica. By conducting a comprehensive genomic analysis and characterizing the strain, we seek to uncover potential new bacterial species that could be valuable for biotechnological applications as pharmaceuticals in the medicinal and veterinary sectors or as bacterial agents for bioremediation.

Materials and Methods

Sampling and Isolation

The water sample was collected at Lystad Bay in Horseshoe Island (67° 49′ 49.3′′ S, 67° 14′ 17.85′′ W) during the 6th Turkish Antarctic Expedition in 2022 using a vacuum filtration system with a cellulose membrane of 0.45 μ m pore diameter. After filtering about 3 It water, the filter was put into a sterile falcon tube and stored at +4°C until the isolation. To isolate bacteria, the filter was suspended in 5 ml sterile Ringer's solution and vortexed vigorously for 5 min, followed by incubation in a rotary shaker for 1.5 h at room temperature. Then, 100 μ l aliquots of the suspension were spread onto Luedemann's agar medium (yeast extract 5 g/l, malt extract broth 15 g/l, starch 10 g/l, glucose 10 g/l, CaCO₃ 2 g/l, NaCl 5 g/l, agar 15 g/l, distilled water 1000 ml, pH 8.6) plates and incubated at 17°C for 30 days. The plates were checked weekly for the purification of the colonies. The subcultured colonies were inoculated on the N-Z-Amine agar medium (glucose 10 g/l, starch 20 g/l, yeast extract 5 g/l, N-Z amine 5 g/l, CaCO₃ 1 g/l, agar 20 g/l, distilled water 1000 ml, pH 7.2) and maintained in the glycerol stock solutions (30%, v:v) at -80°C.

Phylogenetic and Phenotypic Characterization of the Strain

An isolate with red-coloured colonies was selected for further analysis. The genomic DNA of the purified isolate was extracted using PureLink Genomic DNA Isolation Kit (Invitrogen) following collecting the cells grown on N-Z-Amine agar medium at 17°C for 7 days. The 16S rRNA gene from the genomic DNA was amplified using the universal primers 27F (5'-AGAGTTTGATC(AC)TGGCTCAG-3') and 1492R (5'-ACGG(CT)TACCTTGTTACGACTT-3'). The purified PCR products were sequenced in Macrogen Inc. (The Netherlands), and a nearly complete 16S rRNA gene sequence (1469 bp) was deposited in the NCBI GenBank database under accession number OP810936. The pairwise sequence comparisons for the 16S rRNA gene sequences were performed on the EzBioCloud server (https://www.ezbiocloud.net/) (Yoon et al., 2017). For the phenotypic characterization of the strain, the BIOLOG GENIII MicroPlates (Biolog, Hayward, CA) was employed by following the manufacturers' instructions with modifications of the optimum incubation temperature and time for the strains (at 20°C for up to 7 days).

Genome Sequencing and Phylogenomic Analysis

The genome sequencing was performed by MicrobesNG (Birmingham, UK) using an Illumina sequencing technology. The genomic DNA library was constructed using the Nextera XT DNA Library Preparation Kit, and sequencing was performed on the Illumina NovaSeq 6000 platform in 2x150-bp paired-end (PE) mode with a 1000-cycle HiSeq reagent kit. The raw sequence was processed using the KBase Predictive Biology platform (https://www.kbase.us/), and the sequence was uploaded in fastq format. The read quality was assessed using FastQC v 0.11.5 (Brown et al., 2017), and the adaptor sequences specific to the Nextera DNA library were trimmed using Trimmomatic v 0.36 (Bolger et al., 2014). The de novo assembly was conducted using SPAdes v 1.3.4 (Bankevich et al., 2012). The draft genome sequence was submitted to the NCBI GenBank database under the accession number JASISN000000000. The draft genome sequence was annotated on the Rapid Annotations Using Subsystems Technology (RAST) server (https://rast.nmpdr.org/) (Aziz et al., 2008) using RASTtk pipeline (Brettin et al.,

2015).

For a whole-genome-based taxonomic analysis, the genome sequence was uploaded to the Type (Strain) Genome Server (TYGS) available at https://tygs.dsmz.de (Meier-Kolthoff & Göker, 2019). The GBDP approach was employed and accurate intergenomic distances were inferred under the algorithm 'trimming' and distance formula d5 for pairwise comparison of the genome sequences (Meier-Kolthoff et al., 2013). The digital DNA-DNA hybridization (dDDH) values and confidence intervals were calculated using the recommended settings of the GGDC 4.0 (Meier-Kolthoff et al., 2013) after 100 distance replicates were calculated for each genome. For phylogenetic inference, a balanced minimum evolution tree with branch support via FASTME 2.1.6.1, including SPR postprocessing (Lefort et al., 2015), was inferred using the resulting intergenomic distances from the previous step. Branch support in the trees was inferred from 100 pseudobootstrap replicates. The trees were rooted at the midpoint (Farris, 1972) and visualized with PhyD3 (Kreft et al., 2017). For type-based species and subspecies clustering, a 70% dDDH radius around each of the 12 type strains was done as previously described (Meier-Kolthoff & Göker, 2019), and a 79% dDDH threshold was used as previously introduced (Meier-Kolthoff et al., 2014), respectively.

Functional Genome Analyses

The analyses of antibiotic resistance genes and virulence genes were conducted using the Resistance Gene Identifier (RGI) integrated within the Antibiotic Resistance Comprehensive Database (https://card.mcmaster.ca/analyze/rgi [accessed on 13 October 2024]) and the Virulence Factor Database (VFDB) (http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi [accessed on 13 October 2024]), respectively (Alcock et al., 2019; Blin et al., 2023; Liu et al., 2022). The antiSMASH server (https://antismash.secondarymetabolites.org/

[accessed on 13 October 2024]) (Blin et al., 2023) was employed to identify the bioactive secondary metabolite gene cluster. Additionally, prophages were predicated in the genome using the PHASTEST (PHAge Search Tool with Enhanced Sequence Translation) web server (https://phastest.ca/ [accessed on 13 October 2024]). The intact, questionable, and incomplete prophage sequences were defined by score values of >90, 70 to 90, and <70, respectively (Wishart et al., 2023). The CRISPRCasFinder (https://crisprcas.i2bc. paris-saclay.fr/CrisprCas Finder/Index [accessed on 13 October 2024]) server was employed to identify Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-associated (cas) genes sequences. To predict the pathogenicity in human hosts, the PathogenFinder, available at https://cge.cbs.dtu. dk/services/PathogenFinder/, was used (accessed on 13 October 2024) (Cosentino et al., 2013). An in-depth characterisation and visualization of the genome was acquired by the Proksee webserver (https://proksee. ca/) (Grant et al., 2023). In addition, the habitat preferences and ecological distribution pattern of the strain, as well as functional genomic characteristics, were determined by the Protologger webserver (https://protologger.bi.denbi.de/) (Hitch et al., 2021).

Results

Identification and Characterization of the Strain

The pairwise analysis of the 16S rRNA gene sequences showed that strain S3-L1 is a member of the genus *Planococcus* by sharing the highest identity level of 98.8% with *Planococcus kocurii* ATCC 43650^T, followed by *Planococcus faecalis* CECT 8759^T with 98.7%. The rest of the genus shared a relatively low level of 16S rRNA gene sequence identity (<97.7%) with strain S3-L1. The phylogenetic neighbours of strain S3-L1 also originated from cold habitats, i.e. *P. kocurii* was isolated from the skin of North Sea cod *Gadus callarias* (Van Hao & Komagata, 1985) and *P. faecalis*, a carotenoid-producing bacterium, was isolated from stools of Antarctic penguins (Kim et al., 2015). Similar to *P. faecalis*, strain S3-L1 could also produce carotenoid-like pigments.

The physiological and biochemical characteristics of the strain determined by BIOLOG GENIII MicroPlates (Biolog, Hayward, CA) revealed that strain S3-L1 could oxidise α -D-glucose, 3-methyl glucose, acetoacetic acid, D-cellobiose, D-fructose, D-galactose, D-mannitol, D-mannose, D-melibiose, D-sorbitol, D-turanose, glycerol, N-acetyl-D-glucosamine, N-acetyl- β -D-mannosamine and Tween 40 as sole carbon source. In addition, the strain could tolerate 1% NaCl, aztreonam, tetrazolium violet, and lithium chloride.

Genome Features and Phylogenomic Characteristics

The genome was assembled into 54 contigs with 3.7 Mb size and 40.5% G+C content. The genome coverage, N50, and L50 values were 87x, 138.1 kb and 7, respectively. The RAST annotation revealed that the genome of strain S3-L1 encodes 3756 CDS and 65 RNAs. The draft genome of strain S3-L1 was submitted to the NCBI GenBank database under accession number JASISN00000000. The circular genome map was created by the Proksee web server using a suite of tools including Bakta (Schwengers et al., 2021), CARD/RGI (Alcock et al., 2023), CRISPRCasFinder (Couvin et al., 2018), mobileOG-db (Brown et al., 2022) and Phigaro (Starikova et al., 2020) (Figure 1).

The phylogenomic analysis conducted on the TYGS revealed that strain S3-L1 was closely related to *P*. *faecalis* CECT 8759^{T} and *P. kocurii* ATCC 43650^{T} forming a well-supported clade at the periphery of the genome-based phylogenetic tree (Figure 2). The dDDH analysis showed that strain S3-L1 and *P. faecalis*, as well as *P*.



Figure 1. Functional annotation and visualization of the genome of strain S3-L1 by Proksee web tool.

kocurii, belong to the same genomic species (Table 1) as the dDDH values were higher than the 70% threshold for delineating bacterial species. Considering Rule 24b of the International Code of Nomenclature of Prokaryotes and regulating the priority of names and epithets for heterotypic synonyms (Tindall, 2019) strain S3-L1 and *P. faecalis* should be classified as *P. kocurii* (Figure 2).

Genome Features Related to Virulence and Antimicrobial Resistance

In the virulence gene assay, the identified virulence genes are associated with the formation of polysaccharide capsules and lipooligosaccharides (LOS) in various bacterial species. The gtaB gene, encoding UTP-glucose-1-phosphate uridylyltransferase, was found to be involved in polysaccharide capsule biosynthesis in Bacillus thuringiensis, showing an identity of 83.6% with strain S3-L1. Similarly, BC5275, another UTP-glucose-1-phosphate uridylyltransferase, contributes to capsule formation in Bacillus cereus ATCC 14579, with an identity of 81.6%. The galU gene from Bacillus cereus ATCC 10987 also functions in UTPglucose-1-phosphate uridylyltransferase activity was found in the genome of strain S3-L1 by 81.1% identity. Another gtaB gene from Bacillus thuringiensis serovar konkukian strain 97-27 exhibited an identity of 80.2%. The wbjD/wecB gene encodes UDP-Nacetylglucosamine 2-epimerase, important for capsular polysaccharide biosynthesis in Vibrio cholerae O1 biovar El Tor strain N16961, sharing 85.1% identity with strain S3-L1. In Haemophilus influenzae PittGG, the galU gene is responsible for argininosuccinate lyase activity related to LOS production, showing complete identity in the genome of strain S3-L1. Similarly, in Haemophilus influenzae PittEE, the galU gene is also involved in carbon storage regulation linked to LOS biosynthesis,

with full identity. The *galU* gene in *Haemophilus influenzae* 86-028NP encodes UTP-glucose-1-phosphate uridylyltransferase, contributing to LOS biosynthesis with 100% identity. In *Haemophilus influenzae* Rd KW20, the *galU* gene encodes glucosephosphate uridylyltransferase, essential for LOS formation, again with full identity.

The antimicrobial resistance gene analysis showed that the identified resistance genes related to glycopeptide antibiotic resistance within the *vanM* and *vanG* gene clusters. In the strict analysis, the *vanY* gene, part of the *vanM* cluster, was detected using the protein homolog model and is associated with glycopeptide resistance gene clusters. This gene plays a role in antibiotic target alteration and exhibited 34.43% identity with the matching region. Similarly, the *vanT* gene, part of the *vanG* cluster, was also detected using the protein homolog model. It is associated with glycopeptide resistance and contributes to antibiotic target alteration. The matching region for this gene had 33.62% identity, with 54.07% coverage of the reference sequence length.

In silico pathogenicity analysis revealed that strain S3-L1 has a relatively low probability of being a human pathogen (0.29) and input proteome coverage of 0.22%. One matched pathogenic family and seven matched non-pathogenic families were identified during the analysis. The matched pathogenic family is associated with *Listeria monocytogenes*, where a 30S ribosomal protein S19 was identified with an 86.96% identity match. This indicates the potential for pathogenic features, although the low overall probability and proteome coverage suggest limited pathogenicity. For the non-pathogenic families, matches include organisms such as *Lysinibacillus sphaericus, Exiguobacterium, Bacillus thuringiensis*, and *Bacillus halodurans*. These families are associated with functions like vitamin B12-



Figure 2. Phylogenomic tree inferred with FastME 2.1.6.1 from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d5. The numbers above branches are GBDP pseudo-bootstrap support values > 60 % from 100 replications, with an average branch support of 94.9 %. The tree was rooted at the midpoint.

Table 1. Digital DNA-DNA hybridization values between strain S3-L1 and its phylogenetic neighl	nbour in the genus	Planococcus
------------------------------------------------------------------------------------------------	--------------------	-------------

Query strain	Subject strain	dDDH (%)	C.I. (%)	G+C content difference (%)
Planococcus sp. S3-L1	Planococcus faecalis CECT 8759 [⊤]	95.6	[94.2 - 96.7]	0.32
	Planococcus kocurii ATCC 43650 [™]	85	[82.3 - 87.4]	0.4
	Planococcus versutus L10.15 [™]	26.3	[24.0 - 28.8]	1.13
	Planococcus antarcticus DSM 14505 [™]	25.5	[23.1 - 28.0]	2.49
	Planococcus halocryophilus DSM 24743 [™]	25.3	[23.0 - 27.8]	0.63
	Planococcus donghaensis DSM 22276 [™]	25	[22.7 - 27.5]	0.43
	Planococcus soli C23T [™]	23.2	[20.9 - 25.7]	4.28
	Planococcus stackebrandtii DSM 16419 [⊤]	23.2	[21.0 - 25.7]	4.41
	Planococcus notacanthi APC 4016 [™]	22.6	[20.3 - 25.0]	4.73
	Planococcus wigleyi Sa1BUA13 [⊤]	22.6	[20.4 - 25.1]	4.5
	Planococcus koreensis DSM 15895 [™]	18.7	[16.5 - 21.0]	7.05
	Planococcus mcmeekinii DSM 13963 [⊤]	18.6	[16.5 - 21.0]	5.1

*C.I. Confidence Interval

dependent ribonucleotide reductase, gluconate transporter, UPF0374 protein, LSU ribosomal protein, transcriptional regulator, and putative iron-sulfur scaffold protein, all with identity percentages ranging from 80.19% to 86.81%. These matches suggest a variety of environmental or non-pathogenic functions, supporting the idea that strain S3-L1 may not primarily be a human pathogen.

A total of four CRISPR arrays were detected in the genome of strain S3-L1, while three CRISPR-associated clusters were identified (Figure 3). Although no prophages were identified by the Phastest, nine genes

coding for phage-related proteins, including phage replication protein, abortive infection bacteriophage resistance protein, protein Lp1 protein 5, prophage LambdaSa2 and phage integrase, were detected by the RAST annotation.

Secondary Metabolite-coding Gene Clusters

The biosynthetic potential of strain S3-L1 was evaluated by the antiSMASH web server. The genome of strain S3-L1 was found to encode two terpene gene clusters, one of which shows 83% similarity to the carotenoid gene cluster, while the other shows no similarity to any known gene clusters. In addition, the genome annotation on the RAST server revealed that strain S3-L1 encodes genes related to the metabolism of aromatic compounds such as gentisate, salicylate and quinate, implying its potential for biotechnological applications. Strain S3-L1 has genes coding for fumarylacetoacetate hydrolase family proteins responsible for the degradation of aromatic compounds, such as xylenols and cresols, via the gentisate pathway. The catechol branch of the betaketoadipate pathway is represented by 3-oxoadipate CoA-transferase subunits A and B (EC 2.8.3.6) and succinyl-CoA:3-ketoacid-coenzyme transferase Α subunit A and B (EC 2.8.3.5) in the genome of strain S3-L1. In addition, the strain also encodes 3dehydroquinate dehydratase (EC 4.2.1.10) genes related to quinate degradation, implying the potential of strain S3-L1 to have applications in bioremediation purposes to remove aromatic contaminants from the environment.

As some members of the genus *Planococcus* were reported as producers of quorum-quenching enzymes (See-Too et al., 2017), the genome of strain S3-L1 was also analyzed for potential N-acyl homoserine lactonases. The annotation analysis on the RAST revealed that the genome of strain S3-L1 encodes a quorum-quenching lactonase YtnP.

Ecological Distribution and Habitat Preferences

The ecological distribution and habitat preferences analysis based on the comparison of the whole genome and 16S rRNA gene sequences of strain S3-L1 with the

databases of metagenome-assembled genomes and 16S rRNA gene amplicons via the Protologger showed that strain S3-L1 could be detected in all 19 environments in the IMNGS database (Figure 4) while no metagenomic reconstructed genomes (MAGs) were matched to the genome of strain S3-L1. The 16S rRNA gene sequence of strain S3-L1 was detected in 59.7% of rhizosphere metagenomes with a mean relative abundance ratio of 0.45%. As a strain isolated from Antarctic marine water, strain S3-L1 could be detected in 24.2% of marine metagenomes and 6% marine sediment of metagenomes with a mean relatively low level of abundance. Although strain S3-L1 is an environmental isolate, its relative abundance seems higher in humanrelated habitats, as exemplified by the human vaginal and human lung metagenomes (Table 2).

Discussion

Microorganisms are abundantly present in all sorts of environments, including polar ecosystems such as Antarctic marine habitats. They are essential components of the ecosystems since most biogeochemical cycles depend on the microorganisms for functioning the ecosystems. Notably, the marine environment has a prosperous and diversified population of bacteria that produce a massive natural bioactive chemical of economic relevance. These natural compounds have sparked intense interest because of their broad stability and functionality under adverse climatic circumstances. Members of the genus Planococcus have emerged as such bacterial species since they could produce diverse secondary metabolites such as 2-acetamido-2-deoxy- α -d-glucopyranosyl-(1, 2)-



Figure 3. Genome map of strain S3-L1 annotated for the clustered regularly interspaced short palindromic repeats (CRISPR arrays) and CRISPR-associated (Cas) proteins by the Proksee web tool.

β-d-fructofuranose exhibiting stabilizing effect and glucosyl-3,4-dehydro-apo-8-lycopenoate methyl displaying antioxidant activity as well as metabolites related to hydrocarbon degradation and biosurfactant/bioemulsifier secretion (Waghmode et al., 2020). Strain S3-L1 isolated from marine water samples collected from Antarctica Horseshoe Island also encodes genes required for the degradation of aromatic hydrocarbons, reflecting its potential use for bioremediation. As the strain is well-adapted to cold marine water, the strain could cycle nutrients in aquaculture systems using waste products like ammonia and nitrite as a source of energy, and thus, it can be used to clean up aquaculture effluent and reduce the negative impact of the aquaculture systems on the environment.

Carotenoids are members of the isoprenoid family, which includes plants, fungi, algae, and bacteria. Bacteria are recognized by the production of uncommon C30, C45, and C50-backbone carotenoids. In nonphototrophic bacteria, carotenoid synthesis is most likely regulated by light, constitutively or cryptically. Bacterial carotenoid pigments, due to their unique structure and antioxidant characteristics, are the primary agents that protect against the detrimental effects of UV radiation. Carotenoids modulate membrane fluidity and protect bacterial cells from freezing in permanently cold conditions such as Antarctica, where the temperature is generally below zero and does not surpass 15°C throughout the year. In recent decades, there has been an increased interest in discovering novel bacterial carotenoid sources since bacteria are increasingly used in the commercial synthesis of carotenoids (Sayed et al., 2023; Styczynski et al., 2020). Strain S3-L1 is also producing red-coloured pigments, and genomic analysis revealed that the produced pigments might have a carotenoid structure. Considering the strain is a psychrotolerant bacterium growing between 15-25°C, biotechnological applications requiring the production of carotenoid pigments at relatively low temperatures will make the strain a potential microbial source for commercial applications.

Using quorum-quenching as a biocontrol approach reduces the selection pressure on the targeted pathogens and can reduce the development of antimicrobial resistance. The discovery of more



Figure 4. Habitat preferences of strain S3-L1 predicted by the Protologger web server.

Environment	Detection Ratio (%)	Mean Relative Abundance (%)	Standard Deviation (%)
Rhizosphere	59.70	0.45	1.69
Soil	36.80	0.72	1.65
Marine sediment	24.20	0.09	0.45
Human vaginal	21.20	1.56	9.07
Plant	18.90	0.23	0.67
Freshwater	17.80	0.52	3.94
Pig gut	15.10	0.11	0.41
Bovine gut	13.50	0.27	1.15
Wastewater	11.80	0.34	0.92
Activated sludge	11.40	0.13	0.74
Insect gut	7.80	0.21	0.65
Human skin	7.10	0.45	3.08
Coral	7.10	0.29	1.31
Marine	6.00	0.09	0.40
Human lung	3.80	2.55	11.34
Chicken gut	2.90	0.02	0.02
Mouse gut	2.70	0.03	0.07
Human oral	0.70	0.05	0.08
Human gut	1.20	0.05	0.13

 Table 2. Habitat preference and distribution analysis of strain S3-L1 based on the 16S rRNA gene amplicons obtained from the

 IMNGS database by the Protologger web server

effective quorum-quenching agents is vital since bacterial resistance to current antibiotics is rising, and other pathogen-fighting techniques are urgently needed. Polar habitats such as Antarctica have been identified as possible sources of new enzymes with commercial applications. In this context, the finding of psychrotolerant quorum-quenching bacteria with potential use as biocontrol, remediation, or growth promoters would be beneficial (See-Too et al., 2017) and strain S3-L1 encoding a quorum-quenching lactonase YtnP can be considered as a potential antimicrobial agent for medicinal and/or veterinary purposes.

Conclusion

In summary, the genomic characterization of Planococcus strain S3-L1, isolated from the marine waters of Antarctica, demonstrates its strong potential for diverse biotechnological applications. The strain's ability to produce carotenoid pigments, degrade aromatic compounds and encode quorum-quenching enzymes underscores its adaptability to extreme conditions and suitability for practical applicationsspecifically, Planococcus sp. S3-L1 may play an important role in bioremediation, assisting in the breakdown of environmental pollutants, as well as in the commercial production of carotenoid pigments at low temperatures, leveraging its psychrotolerant nature. Additionally, the strain's quorum-quenching activity suggests its potential as an innovative tool in antimicrobial applications, offering an alternative approach to pathogen control. Overall, Planococcus sp. S3-L1 emerges as a valuable resource for environmental and industrial use, particularly in cold and saline habitats, with broad implications for sustainable biotechnological advancements.

Ethical Statement

Not applicable.

Funding Information

This work was supported by The Scientific and Technological Research Council of Türkiye (TÜBİTAK) under Grant 121Z891.

Author Contribution

Hilal Ay: Supervision, Conceptualization, Methodology, Investigation, Data curation, Visualization, Writing–original draft, Writing–review & editing; Sibel Melisa Sahin and Nihed Ajmi: Methodology, Formal analysis, Muhammed Duman and Izzet Burcin Saticioglu: Investigation, Formal analysis, Data curation, Visualization, Writing– original draft, Writing–review & editing.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was conducted under the auspices of the Presidency of the Republic of Türkiye, supported by the Ministry of Industry and Technology and coordinated by the TUBITAK MAM Polar Research Institute.

References

- Alcock, B. P., Huynh, W., Chalil, R., Smith, K. W., Raphenya, A. R., Wlodarski, M. A., Edalatmand, A., Petkau, A., Syed, S. A., Tsang, K. K., Baker, S. J. C., Dave, M., McCarthy, M. C., Mukiri, K. M., Nasir, J. A., Golbon, B., Imtiaz, H., Jiang, X., Kaur, K., ... McArthur, A. G. (2023). CARD 2023: Expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. Nucleic Acids Research, 51(D1), D690–D699. https://doi.org/10.1093/nar/gkac920
- Alcock, B. P., Raphenya, A. R., Lau, T. T. Y., Tsang, K. K., Bouchard, M., Edalatmand, A., Huynh, W., Nguyen, A.-L. V., Cheng, A. A., Liu, S., Min, S. Y., Miroshnichenko, A., Tran, H.-K., Werfalli, R. E., Nasir, J. A., Oloni, M., Speicher, D. J., Florescu, A., Singh, B., ... McArthur, A. G. (2019). CARD 2020: Antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Research, gkz935.

https://doi.org/10.1093/nar/gkz935

Aziz, R. K., Bartels, D., Best, A. A., DeJongh, M., Disz, T., Edwards, R. A., Formsma, K., Gerdes, S., Glass, E. M., Kubal, M., Meyer, F., Olsen, G. J., Olson, R., Osterman, A. L., Overbeek, R. A., McNeil, L. K., Paarmann, D., Paczian, T., Parrello, B., ... Zagnitko, O. (2008). The RAST Server: Rapid Annotations using Subsystems Technology. BMC Genomics, 9(1), 75.

https://doi.org/10.1186/1471-2164-9-75

- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., & Prjibelski, A. D. (2012). SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology, 19(5), 455–477.
- Blin, K., Shaw, S., Augustijn, H. E., Reitz, Z. L., Biermann, F., Alanjary, M., Fetter, A., Terlouw, B. R., Metcalf, W. W., Helfrich, E. J. N., Van Wezel, G. P., Medema, M. H., & Weber, T. (2023). AntiSMASH 7.0: New and improved predictions for detection, regulation, chemical structures and visualisation. Nucleic Acids Research, 51(W1), W46–W50.

https://doi.org/10.1093/nar/gkad344

- Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics, 30(15), 2114–2120.
- Brettin, T., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Olsen, G. J., Olson, R., Overbeek, R., Parrello, B., Pusch, G. D., Shukla, M., Thomason, J. A., Stevens, R., Vonstein, V., Wattam, A. R., & Xia, F. (2015). RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Scientific Reports, 5, 8365. https://doi.org/10.1038/srep08365
- Brown, C. L., Mullet, J., Hindi, F., Stoll, J. E., Gupta, S., Choi, M., Keenum, I., Vikesland, P., Pruden, A., & Zhang, L. (2022). mobileOG-db: A Manually Curated Database of Protein Families Mediating the Life Cycle of Bacterial Mobile Genetic Elements. Applied and Environmental Microbiology, 88(18), e0099122.

https://doi.org/10.1128/aem.00991-22

- Brown, J., Pirrung, M., & McCue, L. A. (2017). FQC Dashboard: Integrates FastQC results into a web-based, interactive, and extensible FASTQ quality control tool. Bioinformatics, 33(19), 3137–3139.
- Castillo, D. J., Dithugoe, C. D., Bezuidt, O. K., & Makhalanyane,

T. P. (2022). Microbial ecology of the Southern Ocean. FEMS Microbiology Ecology, 98(11), fiac123.

- Cavicchioli, R. (2015). Microbial ecology of Antarctic aquatic systems. Nature Reviews Microbiology, 13(11), 691–706.
- Cosentino, S., Voldby Larsen, M., Møller Aarestrup, F., & Lund, O. (2013). PathogenFinder—Distinguishing Friend from Foe Using Bacterial Whole Genome Sequence Data. PLoS ONE, 8(10), e77302.

https://doi.org/10.1371/journal.pone.0077302

- Couvin, D., Bernheim, A., Toffano-Nioche, C., Touchon, M., Michalik, J., Néron, B., Rocha, E. P. C., Vergnaud, G., Gautheret, D., & Pourcel, C. (2018). CRISPRCasFinder, an update of CRISRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. Nucleic Acids Research, 46(W1), W246–W251. https://doi.org/10.1093/nar/gky425
- Dutta, A., Connors, E., Trinh, R., Erazo, N., Dasarathy, S., Ducklow, H. W., Steinberg, D. K., Schofield, O. M., & Bowman, J. S. (2023). Depth drives the distribution of microbial ecological functions in the coastal western Antarctic Peninsula. Frontiers in Microbiology, 14, 1168507.
- Farris, J. S. (1972). Estimating phylogenetic trees from distance matrices. The American Naturalist, 106(951), 645–668.
- Grant, J. R., Enns, E., Marinier, E., Mandal, A., Herman, E. K., Chen, C., Graham, M., Van Domselaar, G., & Stothard, P. (2023). Proksee: In-depth characterization and visualization of bacterial genomes. Nucleic Acids Research, 51(W1), W484–W492. https://doi.org/10.1093/nar/gkad326
- Grattepanche, J.-D., Jeffrey, W. H., Gast, R. J., & Sanders, R. W. (2022). Diversity of microbial eukaryotes along the west Antarctic Peninsula in austral spring. Frontiers in Microbiology, 13, 844856.
- Hitch, T. C. A., Riedel, T., Oren, A., Overmann, J., Lawley, T. D., & Clavel, T. (2021). Automated analysis of genomic sequences facilitates high-throughput and comprehensive description of bacteria. ISME Communications, 1(1), Article 1. https://doi.org/10.1038/s43705-021-00017-z
- Kim, J. H., Kang, H. J., Yu, B. J., Kim, S. C., & Lee, P. C. (2015). Planococcus faecalis sp. Nov., a carotenoid-producing species isolated from stools of Antarctic penguins. International Journal of Systematic and Evolutionary Microbiology, 65(Pt_10), 3373–3378.
- Kreft, Ł., Botzki, A., Coppens, F., Vandepoele, K., & Van Bel, M. (2017). PhyD3: A phylogenetic tree viewer with extended phyloXML support for functional genomics data visualization. Bioinformatics, 33(18), 2946–2947.
- Lay, C.-Y., Mykytczuk, N. C., Niederberger, T. D., Martineau, C., Greer, C. W., & Whyte, L. G. (2012). Microbial diversity and activity in hypersaline high Arctic spring channels. Extremophiles, 16, 177–191.
- Lefort, V., Desper, R., & Gascuel, O. (2015). FastME 2.0: A comprehensive, accurate, and fast distance-based phylogeny inference program. Molecular Biology and Evolution, 32(10), 2798–2800.
- Liu, B., Zheng, D., Zhou, S., Chen, L., & Yang, J. (2022). VFDB 2022: A general classification scheme for bacterial virulence factors. Nucleic Acids Research, 50(D1), D912– D917. https://doi.org/10.1093/nar/gkab1107
- Margolles, A., Gueimonde, M., & Sánchez, B. (2012). Genome sequence of the Antarctic psychrophile bacterium Planococcus antarcticus DSM 14505.

Meier-Kolthoff, J. P., Auch, A. F., Klenk, H.-P., & Göker, M.

(2013). Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics, 14(1), 60. https://doi.org/10.1186/1471-2105-14-60

- Meier-Kolthoff, J. P., & Göker, M. (2019). TYGS is an automated high-throughput platform for state-of-the-art genomebased taxonomy. Nature Communications, 10(1), 2182. https://doi.org/10.1038/s41467-019-10210-3
- Meier-Kolthoff, J. P., Göker, M., Spröer, C., & Klenk, H.-P. (2013). When should a DDH experiment be mandatory in microbial taxonomy? Archives of Microbiology, 195, 413–418.
- Meier-Kolthoff, J. P., Hahnke, R. L., Petersen, J., Scheuner, C., Michael, V., Fiebig, A., Rohde, C., Rohde, M., Fartmann, B., Goodwin, L. A., Chertkov, O., Reddy, T., Pati, A., Ivanova, N. N., Markowitz, V., Kyrpides, N. C., Woyke, T., Göker, M., & Klenk, H.-P. (2014). Complete genome sequence of DSM 30083(T), the type strain (U5/41(T)) of Escherichia coli, and a proposal for delineating subspecies in microbial taxonomy. Standards in Genomic Sciences, 9, 2. https://doi.org/10.1186/1944-3277-9-2
- Murray, A. E., & Grzymski, J. J. (2007). Diversity and genomics of Antarctic marine microorganisms. Philosophical Transactions of the Royal Society B: Biological Sciences, 362(1488), 2259–2271.
- Reddy, G., Prakash, J., Vairamani, M., Prabhakar, S., Matsumoto, G., & Shivaji, S. (2002). Planococcus antarcticus and Planococcus psychrophilus spp. Nov. Isolated from cyanobacterial mat samples collected from ponds in Antarctica. Extremophiles, 6, 253–261.
- Sayed, A., Elbalasy, I., & Mohamed, M. S. (2023). Novel β-Carotene and Astaxanthin-Producing Marine Planococcus sp.: Insights into Carotenogenesis Regulation and Genetic Aspects. Applied Biochemistry and Biotechnology, 195(1), 217–235.
- Schwengers, O., Jelonek, L., Dieckmann, M. A., Beyvers, S., Blom, J., & Goesmann, A. (2021). Bakta: Rapid and standardized annotation of bacterial genomes via alignment-free sequence identification. Microbial Genomics, 7(11), 000685.

https://doi.org/10.1099/mgen.0.000685

See-Too, W. S., Ee, R., Lim, Y.-L., Convey, P., Pearce, D. A., Yin, W.-F., & Chan, K.-G. (2017). AidP, a novel N-Acyl homoserine lactonase gene from Antarctic Planococcus sp. Scientific Reports, 7(1), 42968.

- Signori, C. N., Thomas, F., Enrich-Prast, A., Pollery, R. C., & Sievert, S. M. (2014). Microbial diversity and community structure across environmental gradients in Bransfield Strait, Western Antarctic Peninsula. Frontiers in Microbiology, 5, 647.
- Starikova, E. V., Tikhonova, P. O., Prianichnikov, N. A., Rands,
 C. M., Zdobnov, E. M., Ilina, E. N., & Govorun, V. M.
 (2020). Phigaro: High-throughput prophage sequence annotation. Bioinformatics, 36(12), 3882–3884.
- Styczynski, M., Rogowska, A., Gieczewska, K., Garstka, M., Szakiel, A., & Dziewit, L. (2020). Genome-based insights into the production of carotenoids by Antarctic bacteria, Planococcus sp. ANT_H30 and Rhodococcus sp. ANT_H53B. Molecules, 25(19), 4357.
- Tindall, B. (2019). Rule 24b of the International Code of Nomenclature of Prokaryotes and regulating the priority of names and epithets considered to be associated with heterotypic synonyms. International Journal of Systematic and Evolutionary Microbiology, 69(12), 3980–3983.
- Tonelli, M., Signori, C. N., Bendia, A., Neiva, J., Ferrero, B., Pellizari, V., & Wainer, I. (2021). Climate projections for the Southern Ocean reveal impacts in the marine microbial communities following increases in sea surface temperature. Frontiers in Marine Science, 8, 636226.
- Van Hao, M., & Komagata, K. (1985). A new species of Planococcus, P. kocurii isolated from fish, frozen foods, and fish curing brine. The Journal of General and Applied Microbiology, 31(5), 441–455.
- Waghmode, S., Suryavanshi, M., Sharma, D., & Satpute, S. K. (2020). Planococcus species—an imminent resource to explore biosurfactant and bioactive metabolites for industrial applications. Frontiers in Bioengineering and Biotechnology, 8, 996.
- Wishart, D. S., Han, S., Saha, S., Oler, E., Peters, H., Grant, J. R., Stothard, P., & Gautam, V. (2023). PHASTEST: faster than PHASTER, better than PHAST. Nucleic Acids Research, 51(W1), W443–W450.
- Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H., & Chun, J. (2017). Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and wholegenome assemblies. International Journal of Systematic and Evolutionary Microbiology, 67(5), 1613–1617.