Journal of Plant Growth Regulation (2025) 44:7106–7123
https://doi.org/10.1007/s00344-024-11507-4

Algal Growth and Morphogenesis-Promoting Factors Released by Cold-Adapted Bacteria Contribute to the Resilience and Morphogenesis of the Seaweed *Ulva* (Chlorophyta) in Antarctica (Potter Cove)

Fatemeh Ghaderiardakani[1] · Johann F. Ulrich[1] · Emanuel Barth[2] · Maria Liliana Quartino[3] · Thomas Wichard[1]

Received: 9 July 2024 / Accepted: 20 September 2024 / Published online: 14 October 2024
© The Author(s) 2024

Abstract

Macroalgae are found in a variety of marine vegetation ecosystems around the world, contributing significantly to global net primary production. In particular, the sea lettuce species, i.e., members of the genus *Ulva* (Chlorophyta), are located in many ecological niches and are characterized by excellent adaptability to environmental changes but depend on essential associated bacteria, which release algal growth and morphogenesis-promoting-factors (AGMPFs). Our work investigated the hypothesis that bacteria need to be stress-adapted to provide sufficient amounts of AGMPFs for the growth and morphogenesis of *Ulva* throughout its life cycle, even under severe environmental conditions. Our study thus aimed to understand which bacteria contribute to overcoming a variety of stressors in polar regions. Green macroalgae were collected from Potter Cove, King George Island (Isla 25 de Mayo), Antarctica, to study the associated microbiome and, subsequently, to identify AGMPFs releasing bacteria. Therefore, microbiome analysis was combined with morphogenetic bioassays and chemical analysis, identifying bacteria essential for algal growth under Antarctic conditions. Hereby, axenic cultures of *Ulva compressa* (cultivar *Ulva mutabilis*, Ria Formosa, Portugal), previously developed as a model system for bacteria-induced algal growth and morphogenesis, were inoculated with freshly isolated and cultivable Antarctic bacteria to determine their morphogenetic activity. The exploratory microbiome investigation identified numerous cold-adapted AGMPF-producing bacteria. Unlike the temperate-adapted bacterial strains originally isolated from the *U. mutabilis* holobiont, the cold-adapted isolates *Maribacter* sp. BPC-D8 and *Sulfitobacter* sp. BPC-C4 released sufficient amounts of AGMPFs, such as thallusin and still unknown compounds, necessary for the morphogenesis of the Antarctic *Ulva* even at 2 °C. Our results illustrate the role of chemical mediators provided by bacteria in cross-kingdom interactions under cold conditions within aquatic systems. The newly isolated bacteria will enable further functional studies to understand the resilience of the holobiont *Ulva* and might be applied in algal aquaculture even under adverse conditions. The study highlights the importance of eco-physiological assays in microbiome analysis.

Keywords Antarctica · Algal growth and morphogenesis-promoting factors · Cold adaptation · Chlorophyta · *Maribacter* · Thallusin

Handling Author: Axel Mithöfer.

✉ Thomas Wichard
thomas.wichard@uni-jena.de

[1] Institute for Inorganic and Analytical Chemistry, Friedrich Schiller University Jena, Lessingstraße 8, 07743 Jena, Germany

[2] Bioinformatics Core Facility, Friedrich Schiller University Jena, Leutragraben 1, 07743 Jena, Germany

[3] Department of Coastal Biology, Argentinean Antarctic Institute, Buenos Aires, Argentina

Introduction

Seaweeds (marine macroalgae) are known as "ecosystem engineers" due to their essential roles in global ecology (Jones et al. 1994; Duarte et al. 2005). They are cosmopolitan and have become a source of valuable, sustainable biomass in the food, feed, chemical, and pharmaceutical industries (Charrier et al. 2017; Simon et al. 2022; Juul et al. 2024). Sea lettuce (belonging to the genus *Ulva* of Chlorophyta) is characterized by its adaptability to environmental

changes. It is often found in waters heavily polluted by humans and under extreme temperatures, such as in polar or tropical regions. To survive as established population, macroalgae must have successfully resisted a multitude of stressors, such as cold temperatures, dramatic changes in light, and large doses of UV radiation recently studied by omics approaches (Zhao et al. 2016; Ghaderiardakani et al. 2022; Gu et al. 2022). To understand the effects of these factors, including global warming, on biodiversity or ecosystem features, a greater understanding of the stress mitigation techniques and adaptive processes of these species is needed (van der Loos et al. 2019; Ghaderiardakani et al. 2020). The close relationship between macroalgae and bacteria, particularly in extreme ecosystems such as that of Antarctica, represents an exceptional study system for better understanding of the host-microbiome acclimation to harsh environmental conditions and the associated adaptive mechanisms involved in the functioning of the polar marine ecosystem.

The functional importance of associated microbial communities in other terrestrial ecosystems (mainly plants) and the development of host resilience to various stresses have been the focus of many investigations (Chojak-Koźniewska et al. 2018; de Vries et al. 2020). Similarly, in many ways, marine life depends on symbiotic interactions with microbes. For instance, microbial communities control various aspects of the biology and ecology of algal hosts (Wahl et al. 2012; Egan et al. 2013; Saha et al. 2024), including growth and morphogenesis (Marshall et al. 2006; Spoerner et al. 2012; Wichard 2015), adaptation and acclimation (Dittami et al. 2016; Ghaderiardakani et al. 2020), resilience and health (Abdul Malik et al. 2020; Clements and Hay 2023), as well as reproductive performance and settlement (Callow and Callow 2006). In turn, algae represent a rich source of organic components recognized as potential primary chemical signals that drive the dynamics of bacterial communities within the algal–bacterial alliance (Alsufyani et al. 2017; Seymour et al. 2017; Kessler et al. 2018).

Sampling a holobiont (i.e., an alga and its associated bacteria) from its natural but harsh environment is a first experimental approach to study adaptation strategies. Marine macroalgae in the southern polar region experience very low water temperatures. The water temperature in the Antarctic coastline ranges from approximately − 1.8 °C in winter to a maximum of 5 °C at the northern border of the Antarctic border. Endemic Antarctic species have the lowest temperature requirements for survival worldwide, with a maximum growth temperature of 5 °C and a very low upper survival temperature (10–15 °C) (Wiencke and Dieck 1990; Bischoff-Bäsmann and Wiencke (1996); Eggert and Wiencke 2000). When considering the strategies of macroalgae for adaptation to the lowest temperatures, their associated bacterial community must be considered to understand how life is maintained under such extreme conditions. For example, it has been suggested that carotenoid-producing bacteria could enable survival under the unique environmental conditions in Antarctica, such as the low temperature, drastic changes in light, freezing-thaw events, high doses of UV radiation, and relatively low humidity (Alvarado et al. 2018; Vila et al. 2019). The cross-kingdom interactions and the potential survival strategies involved in stress-moderating interactions have not been systematically investigated in relation to the structure, phylogenetic diversity, and functional role of the microbial communities associated with Antarctic macroalgae. Notably, the surface microbiome differs across macroalgae species but also exhibits variations on the surface of the same species (Burke et al. 2011; Gaitan-Espitia and Schmid 2020). For example, the green macroalga *Ulva australis* had high phylogenetic variability in its microbial composition but only a small amount of functional variability, suggesting that alga-associated microbial communities have a core set of functional genes (Burke et al. 2011). Generally, microbial functional genes are more significant in shaping algal host microbial communities than phylogenetic biofilm composition. Consequently, these functions may be widespread across taxonomic groups (Burke et al. 2011). Furthermore, environmental factors can affect the microbiome function without altering its diversity (Hmani et al. 2024). Thus, several studies suggested that an interplay of selection and random, often temporally ordered processes shapes the microbiome of *Ulva,* inhabiting always an essential "functional core" (Burke et al. 2011; Ghaderiardakani et al. 2017; Kessler et al. 2018; Gaitan-Espitia and Schmid 2020). Our study aims to isolate bacteria that harbor these "core functions" from the Antarctic *Ulva* necessary for its growth.

One of the well-studied model systems for investigating the role of cross-kingdom interactions in the completion of morphogenesis and development is a tripartite model system including the axenic green alga *Ulva mutabilis* and its two associated essential bacteria (*Roseovarius* sp. strain MS2 and *Maribacter* sp. strain MS6) (Wichard 2015; Mantri et al. 2020; Blomme et al. 2023). Without the bacteria, the gametes of *U. mutabilis* develop into a callus-like biomass characterized by slow growth, atypical cell wall formation with protrusions, and a lack of thalli and rhizoids (Spoerner et al. 2012; Grueneberg et al. 2016). However, within the tripartite system, *Roseovarius* sp. strain MS2, via a yet-unknown compound, induces cell division (Wichard 2023), and in turn, these morphogen-producing bacteria benefit from the interaction using the glycerol released by *Ulva* as a primary carbon source (Kessler et al. 2018). The second essential bacterium, *Maribacter* sp. strain MS6, triggers cell differentiation, cell wall development, and rhizoid formation in *U. mutabilis* by releasing the bacterial morphogen thallusin, the only identified algal growth and morphogenesis-promoting factor (AGMPF) in *Ulva* (Wichard et al. 2015; Alsufyani et al. 2020). At minimal concentrations ($EC_{50} = 5$ pmol L^{-1}),

the morphogen thallusin induces rhizoid and cell wall formation (Alsufyani et al. 2020; Dhiman et al. 2022; Ulrich et al. 2022). Thallusin was initially isolated from the bacteria *Cytophaga* sp. and *Zobellia* sp., which promote thallus development in *Monostroma oxyspermum* (Matsuo et al. 2003, 2005). Like plant hormones, thallusin has distinct roles in algal development depending on the recipient species and works synergistically with unknown *Roseovarius*-released factors as an inducer of algal morphogenesis in *Ulva* spp. (Alsufyani et al. 2020).

The model organisms *U. mutabilis* and *U. compressa* were previously considered conspecific (Steinhagen et al. 2019). We will refer to *U. mutabilis* in this study to be consistent with the literature and avoid confusion with prior studies in which natural isolates were predominantly characterized based on physical features (Føyn 1958). To elucidate the complex interactions in the holobiont of *Ulva* and to explore adaptation mechanisms, we developed a holistic top–down approach beginning with surface microbiome analysis to identify cold-adapted bacteria (outlined in Fig. 1). This process involves using morphogenetic *Ulva* bioassays to detect bacteria that release potentially AGMPFs under adverse conditions, followed by quantifying thallusin production in bacterial media exposed to cold temperatures. We hypothesized that *Ulva* could respond adequately to stress if its macroalgal phycosphere microbiome adapted to environmental changes to provide the necessary algal growth-promoting factors.

Methods

Collection Site and Sampling of Macroalgae and Bacteria

The sampling locations were selected in the ice-free region in Potter Cove and Potter Peninsula (Isla 25 de Mayo/King George Island), Antarctica (62°14′ S 58° 31′ W) (Fig. 2A) (Wiencke et al. 1998; Quartino et al. 2005; Fromm et al. 2020). Approximately 50 algal specimens of green algae, such as *Ulva* spp. and *Monostroma* spp., were collected from the Antarctic Specially Protected Area (ASPA 132),

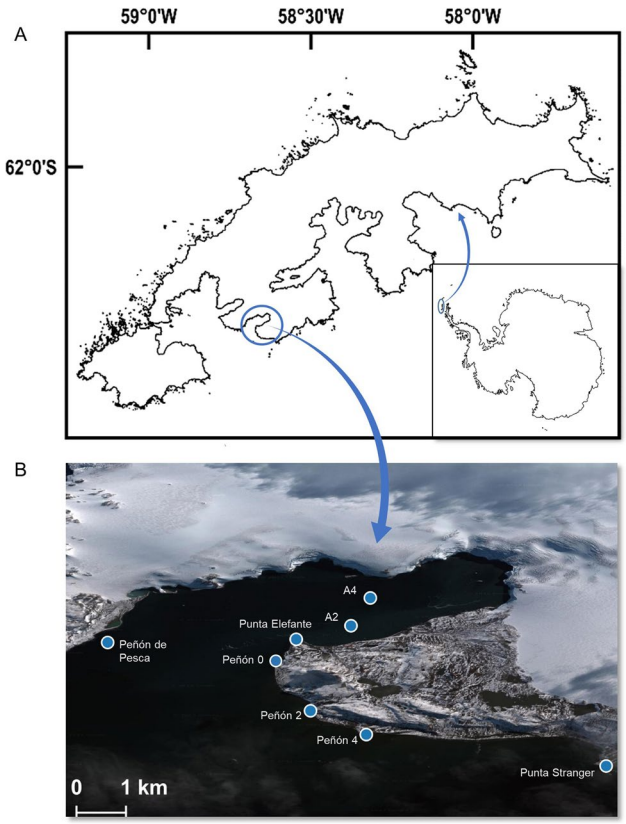


Fig. 2 Sampling sites. **A** Location of the studied sites on Potter Cove and Potter Peninsula on Isla 25 de Mayo/King George Island within the Shetland Archipelago, Antarctica (Potter Cove, Google Maps, 1 January 2023). **B** Eight sampling sites are shown along the coastline and in Potter Cove, including Peñón de Pesca (PDP), Punta Elefante (PE), and Punta Stranger (PS). Satellite image of Potter Cove: Google Earth 2020

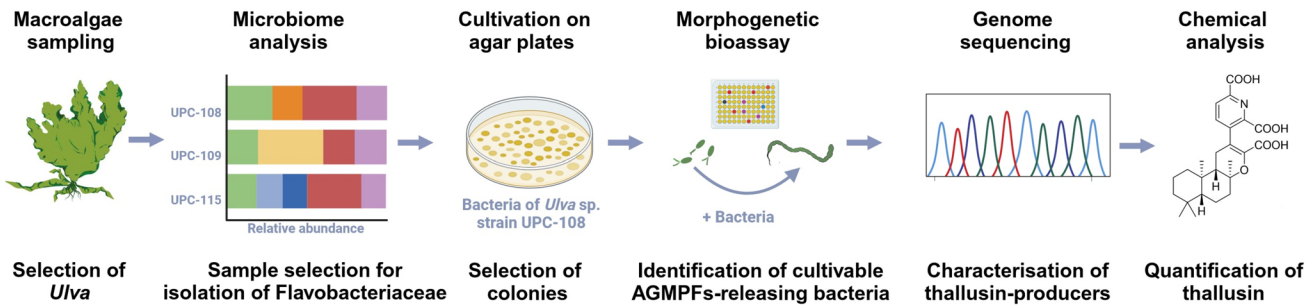


Fig. 1 Workflow. The study combines field surveys, laboratory experiments, and a reductionistic model system to reveal the importance of an adapted microbiome in facilitating symbiotic interactions between the (surface) microorganism and the macroalga *Ulva*. Created in BioRender. Wichard, T. (2024) http://Biorender.com/f02y113

and water samples were collected from the coastline at low tide in January/February 2020 (Fig. 2B). The samples were transported to the Dallmann Laboratory operated by the Alfred Wegener Institute (AWI) and Instituto Antártico Argentino (IAA) adjacent to the Argentinean Carlini Station. Ilicic et al. (2023) reported additional metadata of the Potter Cove, such as temperature and salinity, that were collected during the expedition in 2020.

Taxonomic Classification of the Macroalgae Collected in Potter Cove

The macroalgal specimens were collected from Potter Cove, near the Carlini Station and the intertidal platform further outside the bay on King George Island, to determine the green macroalgal abundance, species composition, and community structure in the habitat (Fig. 3). The samples were frozen and maintained at − 80 °C during shipment to the Alfred Wegener Institute (Bremerhaven, Germany).

For genotyping, a tissue sample (up to 100 mg fresh weight per macroalga) was used for genomic DNA extraction using the GenElute Plant Genomic DNA Miniprep Kit (Merck). Genotyping PCR was performed using PrimeSTAR GXL DNA Polymerase (Takara) in the presence of 5% DMSO (Kwantes and Wichard 2022) with the primer pair designed for *tuf*A (forward: GGNGCNGCNCAAATG GAYGG, reverse: CCTTCNCGAATMGCRAAWCGC) (Famà et al. 2002; Saunders and Kucera 2010). The biosamples were named according to their identification details, such as organism type, location, and identifier, and were archived at the National Center for Biotechnology Information (NCBI; Supplementary Material: Table S1).

Microbiome Analysis of Surface-Associated Bacteria

After systematically checking for sessile organisms, which, if present, were carefully removed with a sterile scraper, the algal tissue was rinsed three times in autoclaved *Ulva* culture medium to remove unattached bacteria from the surface. Three replicates of 200 mg of algal tissue were cut into small pieces, rapidly frozen with liquid nitrogen, and stored at − 80 °C until DNA extraction. According to the manufacturer's protocol, microbial metagenomic DNA was extracted using the DNeasy® Power Soil® Kit (Qiagen, Germany). The primers 515F-Y "new EMP" GTGYCAGCMGCCGCG GTAA and 806RB "new EMP" GGACTACNVGGGTWT CTAAT, which target the V4 region of the 16S rRNA gene, were used for sequencing via the Illumina MiSeq 3 platform (LGC Genomics GmbH, Berlin, Germany). Microbiome analysis was initiated by performing quality control and investigating the raw sequencing reads using FastQC v0.11.9. Quality filtering and trimming were performed using prinseq (version 1.2.4) with the following parameters: -min_qual_mean 20 -ns_max_n 0 -noiupac -trim_qual_right (Schmieder and Edwards 2011). The DADA2 pipeline v1.20.0 (Callahan et al. 2016) in R v4.0.3 was used to process the resulting reads and infer exact amplicon sequence variants (ASVs) from the paired-end reads. The forward and reverse reads were merged to obtain fragments ranging from 220 to 263 nucleotides in length, with 93.8% having a length of 253 nucleotides. The sequences were grouped by 100% similarity to generate unique ASVs (in total 5305), and taxonomic identities were assigned to them using the pretrained naïve Bayes classifier on the DADA2-formatted SILVA database (silva_nr99_v.138.1) (Quast et al. 2013) (Fig. 4; NCBI accession numbers of the raw sequencing data: PRJNA828511, SAMN39202330-SAMN39202384; Supplementary Material: Table S2).

Identification of Bacteria That Release Essential AGMPFs Using the Standardized Model System of *Ulva mutabilis*

Microbiome analysis was accompanied by functional examinations to investigate growth and morphogenesis-promoting traits within the microbiome (Hmani et al. 2024). For this, bacteria were isolated from the surface of the algal samples using sterile swabs. They were streaked onto preprepared marine agar (MA) plates (Roth, Karlsruhe, Germany, marine broth (MB) supplemented with 1% agar) to obtain single colonies (Grueneberg et al. 2016). All the plates were incubated at 8 °C for 3 days. After 1–7 days, 443 distinct colonies were selected and transferred with sterile toothpicks into 96-well plates containing 200 µL of MA in each well.

To evaluate the activity of potential morphogenesis-inducing bacteria, a morphogenetic bioassay was performed using axenic cultures of *U. mutabilis* (Grueneberg et al. 2016). Axenic gametes of *U. mutabilis* (strain FSU-UM5-1) were prepared according to the method established by Califano and Wichard (2018) and inoculated with bacteria isolated from green or red macroalgae collected individually from Potter Cove, Antarctica (section"Collection Site and Sampling of Macroalgae and Bacteria"). As positive controls, the AGMPF-releasing reference strains *Roseovarius* sp. (GenBank EU359909) and *Maribacter* sp. (GenBank EU359911) were inoculated singly and jointly with the axenic cultures (Spoerner et al. 2012; Ghaderiardakani et al. 2017). As a negative control, some wells were randomly retained without bacterial inoculation (only axenic gametes) in each row (Fig. 5). All inoculations and controls were carried out in triplicate. After inoculation, the final optical density of the bacteria was $OD_{620} = 1.0 \times 10^{-4}$, and all the cultures were incubated at 18 °C to fulfill the requirements of the model system with *U. mutabilis*.

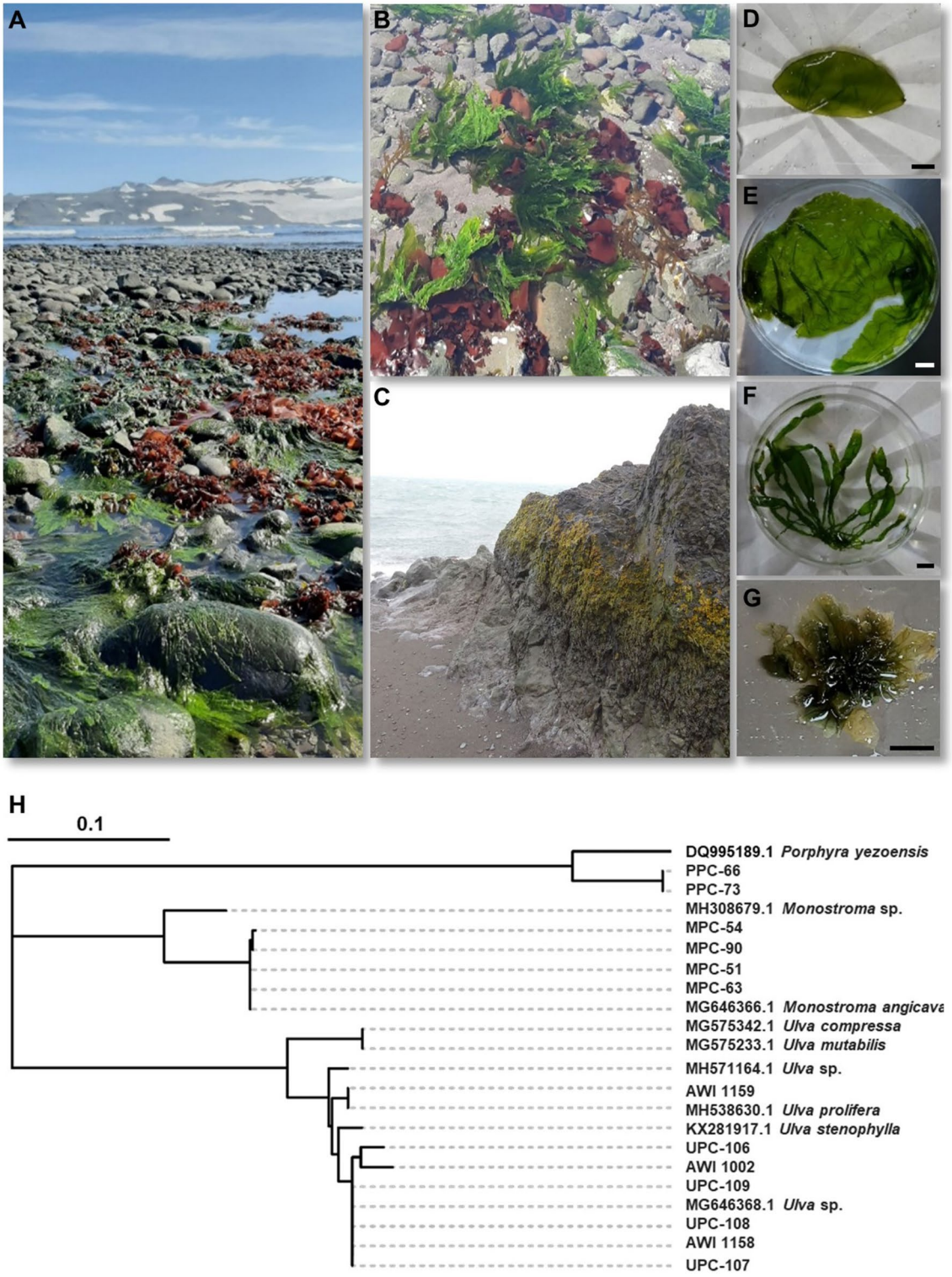


Fig. 3 Diversity of green macroalgae sampled in Potter Cove of King George Island (Antarctica). **A–C** The diversity and abundance of diverse macroalgae (green, red, and brown) are displayed on the coastline. The figures demonstrate that the green algal samples were predominantly distributed in the intertidal zone. Collected green (**D–F**) and red algae (**G**) are shown (scale bars = 1 cm), whereas image **F** refers to UPC-108 and **G** to PPC-66. **H** A phylogenetic tree was inferred from *tuf*A sequences and constructed with the maximum-likelihood method (MEGA 10.1.8; Tamura et al. 2011). The model organism *U. mutabilis* Føyn (Føyn 1958), belonging to "*Ulva-compressa-pseudocuvata*" (Steinhagen et al. 2019), was used as an internal reference, and *Porphyra yezoensis* was used as an outgroup. Photographs **A**, **B**, and **D–F** were used with permission from Fromm et al. (2020)

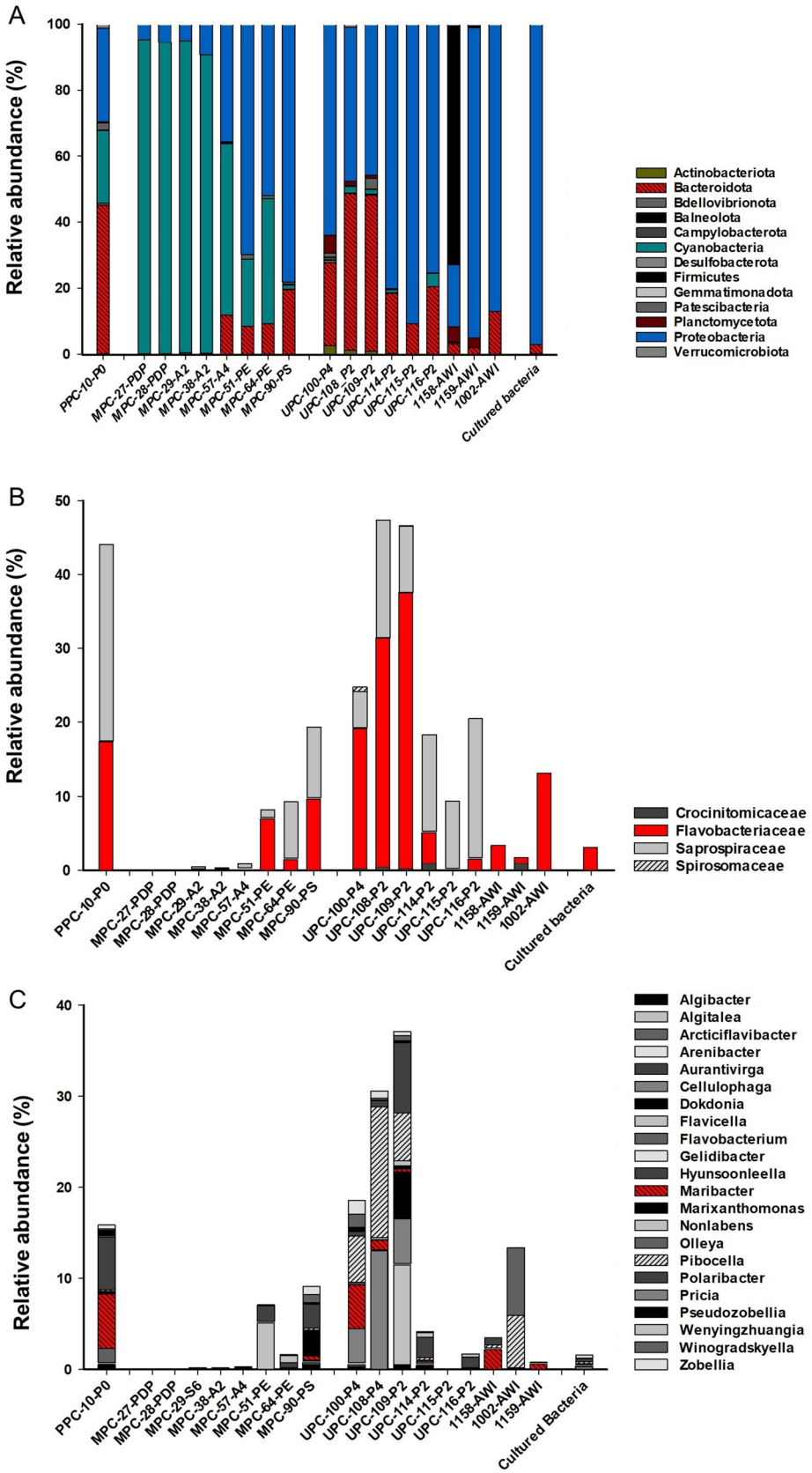


Fig. 4 Relative abundance and prevalence of surface microbes on green macroalgae collected across all the sampling sites of Potter Cove (King George Island, Antarctica). The diversity of the microbiota was compared with that of a mixed sample of bacteria cultivated in marine broth. **A** The relative abundance of whole phyla in the microbiome. **B** The relative abundance of bacteria in the Bacteroidota family. **C** The taxonomic profile of the genera assigned to the family Flavobacteriaceae. (PPC, UPC, MPC: *Porphyra*, *Ulva* or *Monostroma* collected from Potter Cove; AWI: Antarctic *Ulva* sample from the Alfred-Wegener-Institute culture collection). The average of three measurements is shown ($n=3$). A mixture of isolated and subsequent "cultured bacteria" was also analyzed ($n=4$). Species names are arranged alphabetically from top to bottom (*Zobellia*-*Algibacter*). The hatched bars indicate the genera *Maribacter* and *Pibocella*, which were of particular interest in this study

Molecular Identification and Phylogenetic Analysis of the Cultivable Bacteria Collected from the Algal Surface

DNA was extracted from 49 out of 443 cultivable cold-adapted bacterial isolates according to the manufacturer's instructions for the DNeasy Blood and Tissue Kit (Qiagen, Germany) (Grueneberg et al. 2016). To identify the bacteria, partial 16S rDNA sequences (~ 1500 bp) were amplified from these strains using the primer pair 27f (GGG TTT GAT CCT GGC TCA G) and 1390r (ACG GGC GGT GTG TRC AA) (Frank et al. 2008). The preparation of the reaction mixture and amplification of 16S rRNA was performed following the protocol described by Ghaderiardakani et al. (2017). The PCR products were then subjected to forward primer-mediated sequencing using the chain termination method (Eurofins, Germany). The closest homologous sequences in the GenBank database were identified and used to construct the phylogenetic tree.

Taxonomic assignment of the isolated and cultivated bacteria was performed using the Ribosomal Database Project classifier tool (RDP, release 10, http://rdp.cme.msu.edu) at an 80% confidence threshold. Bacterial-type strains representing the closest 16*S* rRNA gene relatives to the sequences generated in this study were identified using the RDP sequence match tool and were included in the phylogenetic inference efforts for taxonomic reliability. For phylogenetic reconstruction, 16*S* rRNA gene sequences from our isolates, their closest type strains, and reference *Ulva*-associated strains were aligned by the SINA web aligner tool within the ARB-SILVA database (Fig. 5; NCBI accession numbers: OR957431-OR957479; Supplementary Material: Table S3).

Bacterial Whole-Genome-Based Phylogeny

The bacterial isolate BPC-D8 (phenocopy of *Maribacter* sp. MS6), which was obtained from the algal surface and tested positive for morphogenetic activity, was cultivated for 4 days in MB medium in sterile polycarbonate flasks (Sarstedt, Germany) at 20 ± 1 °C in natural light with orbital shaking. Genomic DNA was extracted using the cetyltrimethylammonium bromide protocol (William et al. 2004). The DNA sample was sheared in Covaris g-TUBEs, resulting in fragments with a target size of 20 kb. A Pacific Biosciences (PacBio) SMRTbell Express library was prepared according to the manufacturer's instructions using AMPure PB beads for size selection. Microsynth AG (Switzerland) performed library barcoding and pooling/multiplexing, library quality control (Femto Pulse analysis and BluePippin size selection), genome sequencing on a PacBio Sequel sequencer, and subsequent assembly. After subread generation (PacBio single-molecule real-time [SMRT] Link v.10.1) and extraction (SAMtools v.1.14), Filtlong v.0.2.1 was used to filter subreads smaller than 1000 bp. De novo assembly was performed using the Flye assembler (v.2.8.3). The default parameters were applied for all the software packages. Error correction and Flye software enabled the detection of circularity. The taxonomic classification was determined using the Genome Taxonomy Database (GTDB) with GTDB-Tk v1.7.0. The taxonomic method was based on the classification defined by topology and average nucleotide identity (ANI)-based comparisons (https://www.kbase.us/) (Jain et al. 2018; Chaumeil et al. 2020).

Thallusin Quantification in Cold-Adapted Bacterial Cultures

The bacteria were cultivated in 200 mL of HaHa_100 growth medium (Hahnke et al. 2015) on an orbital shaker (230 V Euro Plug, Standard 5000, VWR). The optical density (OD) of the cultures at a wavelength of 620 nm was measured daily using a UV−Vis spectrophotometer (Genesys 10S UV−Vis Spectrophotometer, Thermo Scientific, Waltham, MA, USA). An OD versus cell counts calibration curve was prepared to determine cell density (Ulrich et al. 2022). Thallusin quantification was performed on an ultrahigh-performance liquid chromatography (UHPLC) system linked to a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific). Thallusin was extracted using C_{18} solid-phase extraction followed by a derivatization step with iodomethane to prevent the formation of Fe–thallusin complexes, which would interfere with chromatographic separation (Ulrich et al. 2022).

Results and Discussion

Given the worldwide distribution of green macroalgae, these organisms have managed to withstand a variety of stressors to become the established populations we are familiar with today. To understand the adaptations of *Ulva* to Antarctic conditions, macroalgae were collected from Potter Cove and Potter Peninsula, Isla 25 de Mayo/King George Island (Fig. 2, Table 1). The microbiome of the algal surface was assessed, and the bacteria-releasing AGMPFs were isolated. The production and prevalence of the AGMPF thallusin under cold stress was a particular criterion examined in the bacterial survey.

Collection and Identification of Antarctic Seaweeds in Potter Cove

In the Antarctic summer of 2020, the Antarctic coastline at Potter Cove was covered with brown, red, and, in some


A
B Categories of phenotypes (samples n = 443)
47
18
12
40
326
No morphogenetic activity
Phenocopy of Roseovarius sp. MS2
Phenocopy of Maribacter sp. MS6
New morphotype induced (flatted spherical)
Algicidal activity
C
MG-bioassay
Origin
BPC-A5 M
BPC-A8 M
Psychromonas Antarctica DSM 10704
BPC-H5 U
BPC-D4 P
BPC-G11 P
Pseudoalteromonas aestuarlivivens CECT 8945
BPC-C8 M
Pseudoalteromonas antarctica CECT 4664
BPC-G6 M
BPC-E2 U
BPC-C3 M
BPC-A10 S
BPC-A7 M
BPC-A9 M
BPC-A6 M
BPC-C10 S
BPC-C1 M
BPC-F3 P
BPC-F4 P
Alteromonas addita DSM 15446
BPC-H12 S
Colwellia aestuarii DSM 17314
Marinomonas agarivorans KCTC 52475
BPC-H4 P
BPC-G3 P
BPC-C12 S
Cobetia marina DSM 4741
BPC-D2 S
BPC-C6 S
Psychrobacter aquaticus DSM 15339
BPC-G4 M
BPC-D1 M
BPC-F8 M
Paracoccus aestuarii DSM 19484
BPC-F5 S
BPC-B10 P
Roseovarius sp. MS2
Roseovarius aestuarii DSM 29438
Sulfitobacter brevis DSM 11443
BPC-B7 S
BPC-C4 U,P
BPC-B4 S
Micrococcus antarcticus CCUG 57106
Planococcus antarcticus DSM 14505
BPC-B1 P
BPC-D3 U
Polaribacter filamentus DSM 13964
BPC-H1 M
BPC-E3 U
BPC-H6 P
BPC-C5 M
BPC-B8 P
BPC-B12 P
Maribacter sp. MS6
Maribacter antarcticus NR 044515.1
Pibocella ponti KCTC 12262
Maribacter aquivivus DSM 16478
BPC-D8 U,P
Cellulophaga algicola DSM 14237
BPC-E1 S
BPC-F9 P
BPC-B5 P
BPC-H2 S
BPC-F11 M
BPC-H10
Lacinutrix algicola AKS 293
BPC-G2 M
BPC-E5 M
BPC-C11 S
0.1

◂**Fig. 5** Morphogenetic bioassays with *Ulva mutabilis* and identification of isolated bacteria across all the sampling sites in Potter Cove (King George Island, Antarctica). **A** The figure exemplifies purified *Ulva mutabilis* germlings inoculated with bacteria isolated from green macroalgae collected in Potter Cove (Antarctica). Scale bars = 100 µm. The pictures in the top row on the left (adult) and in the centre (germling) show normal algal development. The destinct incomplete morphotypes are defined in **B** and the main text. The arrows indicate cell wall protrusions that appear in the absence of the *Maribacter*-factor. **B** In total, 443 bacterial isolates from Potter Cove were tested and categorized. **C** The phylogenetic tree (maximum-likelihood method) is based on 50 selected bacteria and their 16S rRNA gene sequences. *Roseovarius* sp. MS2, *Maribacter* sp. MS6, and *Maribacter antarcticus* DSM 21422 were used as reference strains. The results of the morphogenetic (MG) bioassay and the origin of the bacteria are annotated (M: *Monostroma* spp.; U: *Ulva* spp.; P: *Porphyra*; S: surface seawater)

places, green macroalgae (Fig. 3A–C), whereas *Monostroma* spp. were found free-floating in water, and sessile *Ulva* spp. were only occasionally found along the coastline (Figs. 2 and 3). The red alga *Porphyra* sp. was identified at different locations and settled on rocks and stones (Fig. 3C). The surface water temperature at the collection sites varied between 1.7 and 2.5 °C, while the salinity ranged from 33 to 34 PSU.

The green macroalgae discovered were mostly monostromatic (Fig. 3D, E), mixed with a few distromatic foliose specimens (Fig. 3F). Green macroalgal samples (indexed as MPC: *Monostroma* Potter Cove and UPC: *Ulva* Potter Cove) were collected from all the designated sampling stations throughout our excursion, with an emphasis on the distromatic specimens (Fig. 2). Red algae were collected for comparison of the related microbiomes (PPC: *Porphyra* Potter Cove). DNA sequences of the gene *tufA*, encoding the elongation factor Tu, were determined from 10 macroalgal isolates and compared with available sequences in GenBank using the Basic Local Alignment Search Tool.

TufA sequences revealed that MPC isolates #54, #90, #51, and #63 were closely related to *Monostroma angicava* (Fig. 3D, F). In contrast, UPC isolates #106, #109, #108, and #107 were phylogenetically close to *Ulva* strains such as *Ulva linza* and *Ulva prolifera* (Fig. 3H). Moreover, *tufA* sequences of the collected green macroalgae aligned with those of strains isolated near the Great Wall Station on King George Island in 2014 (*Ulva* sp.: MG646368.1; *Monostroma* sp.: MG646366.1).

Interestingly, UPC-106 and UPC-108,107 were phylogenetically close to AWI isolates #1002 and #1158, which were collected 30 years ago in the same region (AWI #1002 was originally taxonomically attributed to *Ulva bulbosa*).

Approximately 120–130 macroalgal species have been recorded in Antarctica, mainly from the western Antarctic area along the peninsula (Wiencke and Clayton 2002). Interestingly, a relatively high proportion of marine flora endemism has been identified in this area (Wiencke and Clayton 2002). The green algae reported from the region between King George Island and Anvers Island include *Prasiola crispa* in the supralittoral zone and other species, such as *Urospora penicilliformis*, *Ulothrix* spp., *Ulva bulbosa,* and *Acrosiphonia arcta*, which were found several times on rocks or in tidal pools approximately 30 years ago (Wiencke and Clayton 2002; Wiencke et al. 2007), but the species differed from the *Monostroma* and *Ulva* strains isolated in this study. The bacteria on the macroalgal surface of those isolates were analyzed to identify candidate algal symbionts releasing AGMPFs.

Microbiome Analysis of Macroalgae Collected from Antarctica as a Guide for the Isolation of AGMPF-Producing Bacteria

To provide a comprehensive overview of the bacteria associated with green macroalgae, the surface microbiomes of the algal specimens collected from the various sampling sites in this work (Fig. 2) were compared with the microbiomes of *Ulva* species collected from Antarctica but maintained for approximately 30 years in the culture collection of the Alfred-Wegener Institute (AWI, Bremerhaven, Germany). The Shannon alpha diversity index was affected differentially depending on the locations and macroalga harvested (Fig. S1). The surface microbiomes of *Ulva* collected from coastal areas generally exhibited higher diversity than those from offshore.

The microbial communities found on the surface of *Ulva* in Potter Cove were primarily composed of the phylum Proteobacteria, with Bacteroidota being the second most dominant group (Fig. 4A; Table S4). This observation aligns with previous research conducted in different locations, which also found taxonomically similar dominant microbial communities associated with *Ulva*. (e.g., Friedrich 2012; Califano et al. 2020; van der Loos et al. 2022; Nguyen et al. 2023). Interestingly, *Ulva* and *Monostroma* differed significantly, particularly in the presence of cyanobacteria (Fig. 4A).

Moreover, the comparison of the native surface microbiomes with the mix of 443 isolated and cultivable bacteria (see next section "Bioassay-Guided Classification of Cold-Adapted Bacteria Based on their Induction of Morphogenesis in Ulva mutabilis Føyn"), which constituted only a tiny percentage of the total diversity of the microbiome in general (Fig. 4A), confirmed the prevalence of easily cultivable Proteobacteria (Grueneberg et al. 2016).

Interestingly, the native microbiome contained diverse families (> 3%) within Bacteroidota (Fig. 4B, Table S5), including Flavobacteriaceae, some genera of which, such as *Maribacter* and *Zobellia*, can release the morphogen thallusin potentially (Matsuo et al. 2005; Weiss et al. 2017). Among them, the abundance of Flavobacteriaceae

was greater in the microbiome of *Ulva* samples collected from sites P2 and P4 (UPC-100-P4 (18.5%), UPC-108-P4 (30.5%), and UPC-109-P4 (37.1%)) than in the microbiome of the samples of *Monostroma* spp. attached to the rocks. (Fig. 4B, C). In addition to the *Ulva* microbiome, the *Porphyra* microbiome also showed a high abundance of Flavobacteriaceae. Therefore, *Ulva* and *Porphyra* were selected for a more detailed microbiome analysis of Flavobacteriaceae.

In total, 22 different genera were assigned to Flavobacteriaceae (Fig. 4C, Table S6), which were negligible in the free-floating *Monostroma* compared with *Ulva* or *Porphyra* (Fig. 4B). The abundances of Flavobacteriaceae were greater in sessile specimens, independent of the macroalga taxon, than in free-floating *Monostroma* species. In future research, the reasons for the dominance of Cyanophyceae and the absence of Bacteroidota on the surface of free-floating *Monostroma* compared to sessile species should be investigated (Fig. 4A).

The associated microbiome of *Ulva* isolate UPC-109-P2 included various genera of Flavobacteriaceae, such as *Algitalea* (10.8%), *Polaribacter* (7.6%), *Dokdonia* (5.1%), *Pibocella* (5.3%), and *Cellulophaga* (4.9%), as well as additional genera such as *Maribacter*, with abundances less than 1% (Fig. 4C). As *Maribacter* and related genera are essential for *Ulva* growth and morphogenesis, they must be considered part of the core microbiome of *Ulva* (Fig. 4C) (Weiss et al. 2017; Ulrich et al. 2022). For example, the genera *Pibocella* and *Maribacter* were abundant in *Ulva* samples, regardless of whether they were freshly isolated (UPC isolates) or cultivated (AWI isolates). *Maribacter* and *Pibocella* were dominant in many samples (UPC-108, UPC-109 PPC-10-P0, or 1002-AWI) that attracted our interest and, to a lesser extent, in *Monostroma* (MPC-90-PS). Therefore, guided by the microbiome data, we selected suitable algal isolates promising a high proportion of AGMPF-releasing bacteria and transferred them to agar plates by streaking from the algal surface. We hypothesized that some bacterial isolates could phenocopy *Maribacter* sp. and release AGMPFs such as thallusin.

Changes in the microbiome associated with a specific trait, such as thallusin production by *Maribacter* spp. suggest the presence of a cold-adapted "core microbiome" that could promote *Ulva* growth and morphogenesis. This core microbiome, a common set of bacteria or genes among a majority of host specimens within a particular habitat (Turnbaugh et al. 2007), is essential for bacterial–macroalgal interactions. Importantly, core bacterial communities vary along an environmental salinity gradient (Hernández et al. 2019; van der Loos et al. 2022) or in algal aquacultures (Califano et al. 2020). Numerous biotic and abiotic factors (Abele et al. 2017; Krucon et al. 2021; Galván et al. 2023) or even a single significant temperature anomaly (Ilicic et al. 2023) can cause alterations in the composition of microbial communities in ponds and offshore waters of the Potter Cove. Therefore, shared functional genes rather than taxonomic entities are considered the key criteria underlying the structure of the "functional core" (Burke et al. 2011; Risely 2020) and plays a vital role in ecosystem functioning or substantially impact host fitness and flexibility (Shade and Handelsman 2012). For instance, species of the genera *Sulfitobacter* and *Paracoccus* are known as *Roseovarius*-equivalent species with the ability to produce AGMPFs (i.e., cell division-promoting factors) for supporting *Ulva* development (Ghaderiardakani et al. 2017). In contrast, the genus *Maribacter* and its thallusin production cannot be simply replaced by another bacterial family (Weiss et al. 2017). We thus aimed to isolate strains under cold stress that serve as phenocopy of Maribacter sp. MS6. The macroalgae with the highest abundance of Flavobacteriaceae, UPC-108, UPC-109, and PPC-10-P0, were thus selected according to the microbiome analysis. The omnipresent and widespread activity of the unknown *Roseobacter* factor was investigated in parallel using morphogenetic bioassays.

Bioassay-Guided Classification of Cold-Adapted Bacteria Based on their Induction of Morphogenesis in *Ulva mutabilis* Føyn

Bacterial isolates ($n = 443$) were successfully isolated, cultivated, and screened for the ability to induce cell division and differentiation to establish a novel tripartite community of *Ulva* with cold-adapted bacteria. The microbiome analysis of a mixed sample of all 'cultured bacteria' revealed the dominance of Proteobacteria and, to a lesser extent, Bacteriodota dominated by the Flavobacteriaceae, which both harbor candidate genera for AGMPF production (Fig. 4A, Table S4).

As axenic cultures of the cold-adapted strain UPC-108 were unavailable, axenic cultures of *U. mutabilis* were used under standardized conditions (Grueneberg et al. 2016). Phenotypic analysis of *U. mutabilis* in the presence of cold-adapted bacteria was carried out 3 weeks after inoculation. The following categories (I–V) of bacterium-dependent algal phenotypes, which were originally defined by Grueneberg et al. (2016), were observed (see Fig. 5A,B, Table 2):

(I) Bacteria showed "no morphogenetic activity" and the algae developed a typical callus-like morphology with a lack of adherence and were covered with bubble-like protrusions due to distortions in the exterior cell wall (yellow circle).
(II) Bacterium-induced cell division was observed, such as with the reference strain *Roseovarius* sp. MS2 (magenta circle).
(III) Bacteria, like the reference strain *Maribacter* sp MS6, elicited cell differentiation, rhizoid and cell wall formation (red circle).

(IV) Bacteria showed "algicidal activity" (black circle).
(V) Bacteria induced a "new morphotype," which was characterized by a flat and incomplete thallus (blue circle).

Strains of categories II and III are the most intriguing in this study, as they are candidate strains for mitigating cold stress via bacterial–algal interactions (Table 2).

Most of these bacterial strains ($n = 326$), isolated mainly from *Porphyra* spp. and *Monostroma* spp., had no morphogenetic activity (category I), and only 40 species could partially promote growth, but they did not solely elicit a complete morphotype (category II: phenocopy of *Roseovarius* sp. MS2). Forty-seven isolates induced rhizoid and cell wall formation, potentially due to the release of thallusin (category III: phenocopy of *Maribacter* sp. MS6). In contrast, 12 isolates adversely affected *Ulva* germination (category IV), and 18 isolates induced the rarely observed flattened morphotype (category V) (Fig. 5B). In general, bacteria that phenocopied *Maribacter* sp. MS6 inhabited the algal surfaces (Table 2). The *Ulva* bioassay-guided approach is the first step in obtaining the initial indication of bacteria-dependent morphogenesis in *Ulva* or algicidal activities. The method can serve as a foundation for further studies investigating the underlying traits of bacteria involved in *Ulva* development in a changing environment and their strategies for adapting to stress (Ghaderiardakani et al. 2022).

Identification of the Isolated Bacteria

Fifty out of the 443 isolated bacteria were selected, depending on the morphotypes that they induced, for 16S rRNA-based phylogenetic identification, as determined by the *Ulva* bioassays. *Roseovarius* sp. MS2, *Maribacter* sp. MS6 and *Maribacter antarcticus* DSM 21422 were used as internal controls during sequencing.

Bacterial identification revealed that the isolated bacteria were dominated by *Pseudoalteromonas* spp., which does not confer morphogenetic activity (Fig. 5C). The isolates with the ability to induce cell differentiation and rhizoid formation (category III) were identified as *Lutibacter* sp. (BPC-D3) and *Winogradskyella s*p. (BPC-B5). Importantly, the isolate BPC-D8 neighbored *Maribacter* sp. MS6 and *Pibocella* sp., which are candidates for thallusin production. In contrast, as previously shown, the isolated *Algibacter* species (BPC-H2) were not morphogenetically active (Weiss et al. 2017). Furthermore, the isolates BPC-C4 and BPC-B10, closely related to *Sulfitobacter brevis* DSM 11443 and *Paracoccus aestuarii* DSM 19484, induced cell division and growth (category II) (Fig. 5C). These yet-unknown AGMPFs are produced by a range of bacteria, such as *Roseovarius* sp. strain MS2, *Sulfitobacter* sp. strain MS3, *Halomonas* sp. strain MS1, and *Paracoccus* sp. (Grueneberg et al. 2016; Ghaderiardakani et al. 2017). With the newly isolated Antarctic bacteria, *U. mutabilis* developed its characteristic morphotype after being exposed to BPC-D8 (phenocopy of *Maribacter* sp. MS6) and BPC-C4 (phenocopy of *Roseovarius* sp. MS2) (Fig. 6).

Whole-Genome Sequence of the Novel Cold-Adapted Thallusin-Producing Bacteria

The bacterial isolate BPC-D8 was subjected to additional phylogenetic analysis using PacBio sequencing technology. The draft genome of isolate BPC-D8 was assembled into one contig (4.7 Mbp), with a G + C content of 34.63% and an N_{50} value of 4,783,464 bp (Table S7, NCBI accession number: CP128187). Initial 16S rRNA gene-based sequencing indicated that the isolate was most closely related to *Maribacter aquivivus* and *Pibocella ponti*. Indeed, recent improvements in genomics and taxonomic assignments, such as the application of comparisons and standardized taxonomic classifications based on the ANI using GTDB-Tk (Chaumeil et al. 2020), have further demonstrated that the isolate is more closely affiliated with the suggested type-strain *Maribacter aquivivus* DSM 16478 (Fig. 7). According to GTDB-Tk-v1.7.0 analysis, the ANI between BPC-D8 and *M. aquivivus* (GCA_900142175.1) was 90.55%, based on an aligned fraction of 0.82 (proportion of regions displaying significant similarity) (Fig. 7).

The flavobacterial genus *Pibocella* sp. was first described by Nedashkovskaya et al. (2005) as a marine, heterotrophic, Gram-negative, aerobic, yellow-pigmented, motile-by-gliding bacterium that was isolated from the green alga *Acrosiphonia sonderi* collected from the Sea of Japan. As determined by 16S rRNA gene sequence analysis, the new bacteria were assigned to the family Flavobacteriaceae, and the similarity values of the new isolate and its close relatives *Maribacter aquivivus*, *Maribacter orientalis*, and *Maribacter ulvicola* were 95.8, 95.7, and 95.0%, respectively. Due to the low levels of sequence similarity between this isolate and the other known members of the Flavobacteriaceae family, *Pibocella ponti* gen. nov., sp. nov. was introduced (Nedashkovskaya et al. 2005). However, the taxonomic classification remains controversial, particularly at the genus and species levels (Bowman 2017; García-López et al. 2019). Although an inclusive taxonomic analysis on the classification of bacteria phylogenetically affiliated with the phylum Bacteroidota using a comprehensive set of aligned full-length 16S rRNA gene sequences nested *Pibocella* sp. within the *Maribacter* clade, a taxonomic revision for this genus using cutting-edge advanced genome sequences has been suggested (García-López et al. 2019). Our whole-genome analysis suggested the classification of BPC-D8 into the *Maribacter* clade (Fig. 7), and the strain was thus named

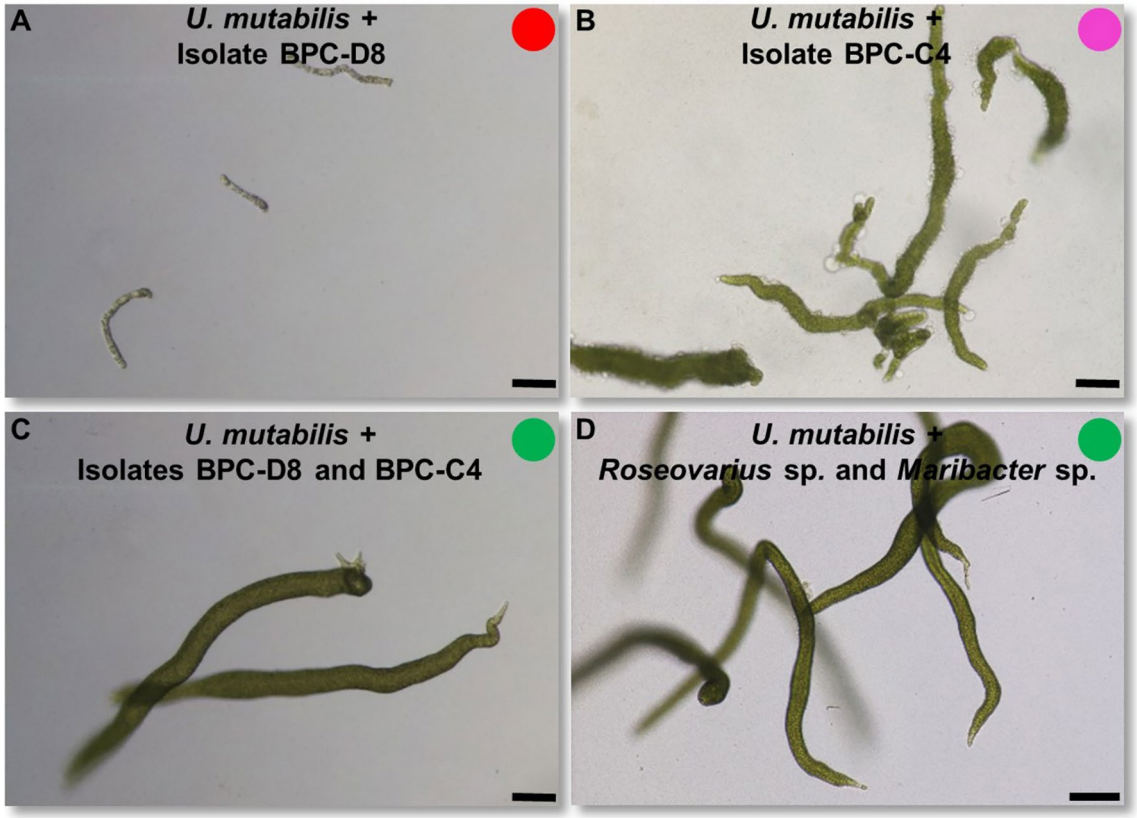


Fig. 6 A combination of two Antarctic bacteria induces the complete morphogenesis of *Ulva mutabilis*. Comparison of the bacterium-induced morphogenesis of *U. mutabilis* through Antarctic bacteria (**A–C**) and temperate-adapted bacteria (**D**) isolated by Spoerner et al. (2012). The combination of isolate BPC-D8 (phenocopy of *Maribacter* sp. MS6, red circle, **A**) and isolate BPC-C4 (phenocopy of *Roseovarius* sp. MS2, magenta circle, **B**) recovers the entire algal morphogenesis (green circle, **C**) in comparison with the positive control (**D**). The color code refers to Fig. 5B. Scale bar = 100 µm

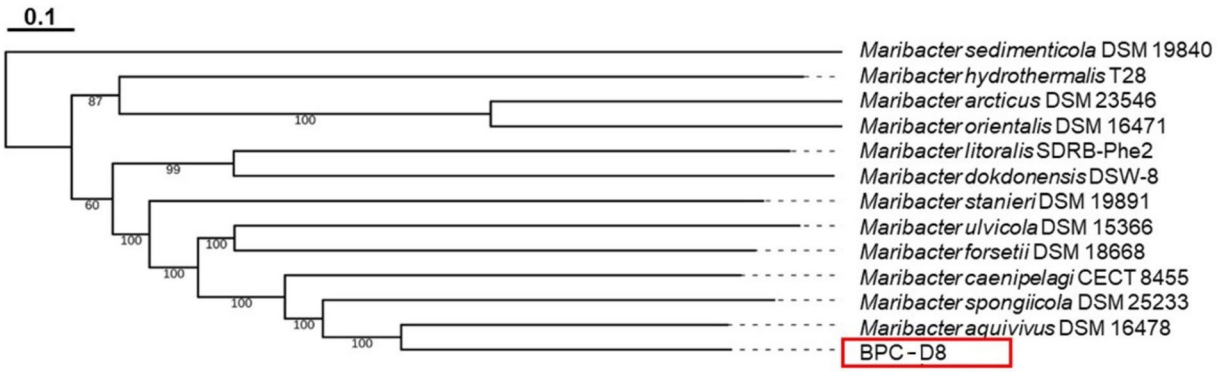


Fig. 7 Phylogenetic analysis of the whole genome of the bacterial isolate BPC-D8. The strain was compared with the type-strain genomes of publicly available genome sequences of *Maribacter* spp. The numbers beside each branch represent the bootstrap values from 100 replications

Maribacter sp. strain BPC-D8. Based on the phylogenetic order, it was likely that this isolate produces thallusin (Weiss et al. 2017), which was subsequently investigated by targeted chemical analyses.

Growth of AGMPF-Releasing Bacteria and Determination of Thallus Growth Under Cold Conditions

Symbiotic bacteria such as *Roseovarius* sp. MS2 and *Maribacter* sp. MS6 release morphogenetic AGMPFs that

Table 1 Sampling site characteristics at Carlini Station, Potter Cove, Isla 25 de Mayo/King George Island

Location	Sample #	Latitude, longitude	Date of sampling	Habitat	Collected macroalgae
A2	29, 38	S 62° 14′ 3.12″ W 58° 40′ 3.00″	01/02/2020	Bay (> 5 m depth)	*Monostroma* spp. (floating)
A4	57	S 62° 13′ 43.68″ W 58° 39′ 49.32″	04/02/2020	Bay (> 5 m depth)	*Monostroma* spp. (floating)
Punta Elefante (PE)	51, 54, 63, 64	S 62°14′12.8″ W 58°40′41.5″	04/02/2020	Coastal water	*Monostroma* spp. (sessile)
Peñón 0 (P0)	10, 66, 73		29/01/2020	Rocks	*Porphyra* spp. (sessile)
Peñón 2 (P2)	106, 107, 108, 109, 114, 115, 116	S 62°15′02.9″ W 58°40′31.4"	09/02/2020	Coastal water	*Ulva* spp. (sessile)
Peñón 4 (P4)	100	S 62°15′19.4″ W 58°39′52.2″	09/02/2020	Coastal water	*Ulva* spp. (sessile)
Punta Stranger (PS)	90	S 62° 15′ 41.76″ W 58° 37′ 4.08″	08/02/2020	Coastal water	*Monostroma* spp. (floating)
Peñón de Pesca (PDP)	27, 28	S 62°14′ 16.5″ W 58° 42′ 16.5″	30/01/2020	Bay (> 5 m depth)	*Monostroma* spp. (floating)

facilitate proper morphogenesis of *Ulva* (Kessler et al. 2018). These bacterial metabolites are essential and may improve algal performance under stress. As a result, we propose that AGMPF and thallusin production could be important examples of a holobiont environmental stress response (Fig. 8). The investigated isolates *Maribacter* sp. MS6 and BPC-D8 were grown at low temperatures. Presumably, because of cold stress, the growth of the explored strains varied greatly (Fig. 8A). The growth of *Maribacter* sp. BPC-D8 began 12 days after inoculation and reached the stationary phase ($OD_{620} = 0.24$) after 24 days, while the growth of *Maribacter* sp. MS6 began after 28 days and reached the stationary phase ($OD_{620} = 0.2$) after 37 days. The growth rates (μ) during the exponential phase of these two bacteria were comparable at 2 °C (for *Maribacter* sp. MS6: $\mu = 0.01 \pm 0.0002\ h^{-1}$; for *Maribacter* sp. BPC-D8: $\mu = 0.009 \pm 0.001\ h^{-1}$) and approximately ten times lower than that at 20 ± 1 °C (Ulrich et al. 2022). Interestingly, the temperate-adapted strain *Roseovarius* sp. MS2 did not grow at 2 °C (Fig. 8B); thus, it cannot provide the essential AGMPFs for *Ulva* growth.

Using mass spectrometry coupled with liquid chromatography, the effective concentration of thallusin was quantified in the culture medium of cold-adapted and temperate *Maribacter* strains at 2 °C. Samples were collected early in the exponential growth phase ($OD_{620} = 0.05$). Due to the increase in thallusin concentration with cell density and accumulation in the medium along with bacterial growth, the thallusin concentration was normalized to cell number and time (0.7 amol $cell^{-1}\ h^{-1}$) (Fig. 8C). Considering the high biological activity of thallusin (Ulrich et al. 2022), the fast production of local high concentrations of thallusin, which was provided by the cold-adapted BPC-D8 and not by the temperate-adapted strain (Fig. 8C), is more ecologically relevant in biofilms on the algal surface (Wahl et al. 2012) than in the culture medium. The prolonged lag phase of the temperate-adapted strain following the change to cold temperature, along with a reduction in thallusin synthesis, prevents the initiation of germination for the released *Ulva* gametes or zoids under Antarctic conditions. In future studies, the hypothesis of whether bacteria can support the transition of *Ulva* from a warm-temperate zone to an Antarctic zone and vice versa can be tested (Fig. 8D).

Table 2 Overview of the determined morphogenetic activities (categories I-V) of the isolated bacteria using the model system *Ulva mutabilis* as bioassay

Origin of the bacteria	Axenic morphotype (I)	Phenocopy of *Roseovarius* sp. MS2 (II)	Phenocopy of *Maribacter* sp. MS6 (III)	Algicidal mode of action (IV)	New flatted morphotype (V)
Porphyra spp.	154	15	13	4	5
Monostroma spp.	131	16	30	6	11
Ulva spp.	26	5	4	1	0
Sea water	15	4	0	1	2
Total	326	40	47	12	18

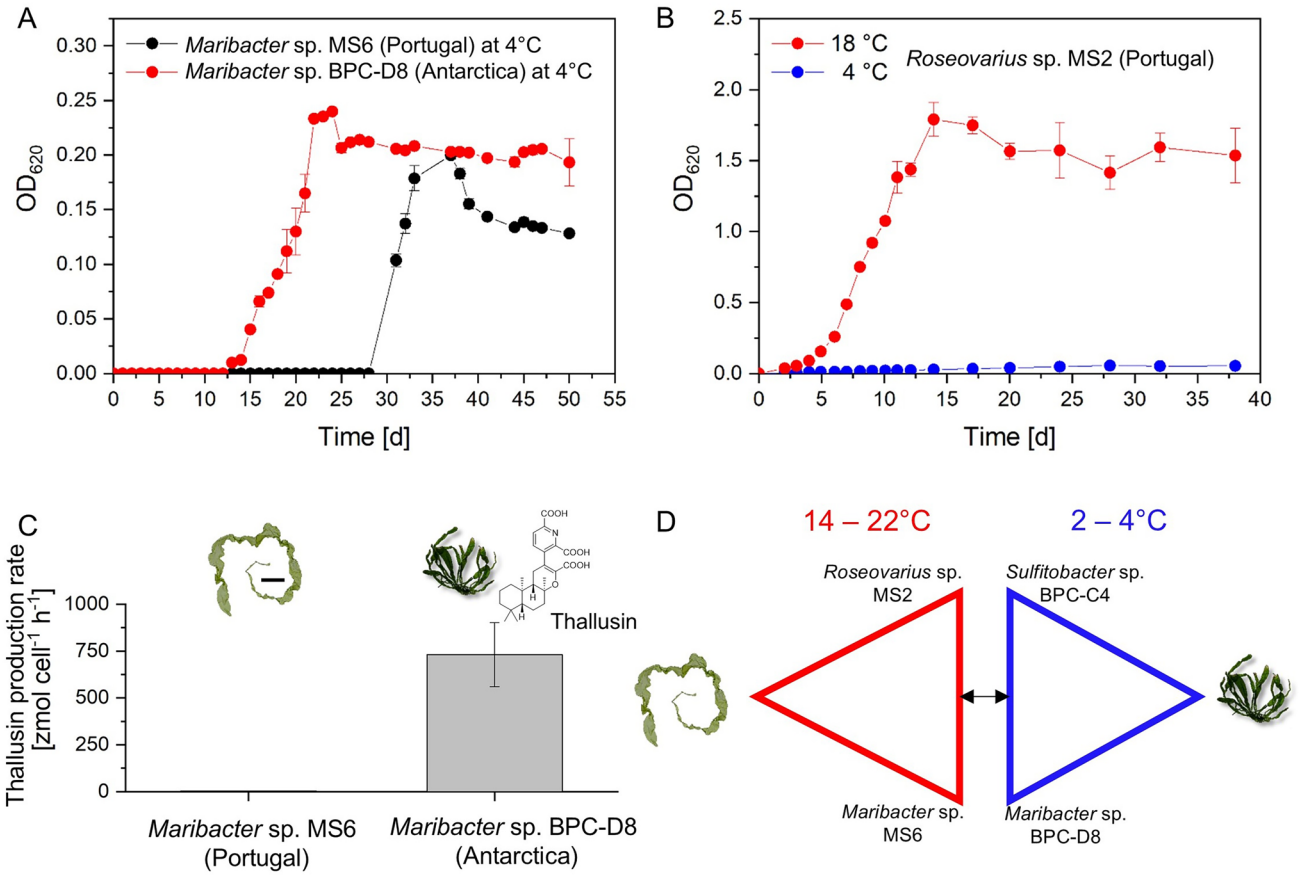


Fig. 8 Bacterial growth and thallusin production promote algal growth and morphogenesis. **A** *Maribacter* sp. MS6 and the isolated *Maribacter* sp. BPC-D8 strains were cultivated in HaHa_100 growth medium for 7 weeks and maintained under cold conditions (2–4 °C). **B** *Roseovarius* sp. MS2 did not grow at 4 °C, only at 18 °C, but the isolated *Sulfitobacter* sp. BPC-C4 could grow on Antarctic temperatures. Both strains were cultivated in marine broth. **C** Following the long lag phase, the temperate-adapted *Maribacter* sp. MS6 grew at 2–4 °C **A**, but the rate at which thallusin was produced was negligible. On the contrary, bacteria of the BPC-D8 isolate released thallusin at a high rate at 2–4 °C at low bacterial density (OD_{620} = 0.05). **D** Proposed novel tripartite model system with *Ulva* cultivated at 2–4 °C and two essential algal growth and morphogenesis-promoting bacteria, *Maribacter* sp. BPC-D8 and *Sulfitobacter* sp. BPC-C4, collected on King George Island (Potter Cove) in Antarctica. Scale bar = 1 cm

Limitations of the Study

While our work focused on the identification of AGMPF-producing bacteria, seasonal studies will contribute to the understanding of changes in the dynamics of the macroalgal phycosphere microbiome (Lu et al. 2023) and the release of growth-promoting factors under various nutritional situations and in a changing environment. Thallusin-producing bacteria were found in red and green algae, which suggests intriguing eco-physiological functions. However, functional studies with *Porphyra* were not performed, because axenic cultures must be established first (Califano and Wichard 2018). Furthermore, the regulation of gametogenesis in isolated Antarctic *Ulva* species should be intensively studied as a prerequisite for achieving axenic cultures of *Ulva* sp. strain BPC-108 under controlled conditions and propagation of gametophytes similar to those of *U. mutabilis* (Stratmann et al. 1996). Finally, our study did not participate in the controversial taxonomic reclassification of *Monostroma* (Cui et al. 2022). Therefore, further phylogenetic studies are needed.

Conclusions

Our work contributes to key questions related to stress ecology in marine ecosystems, which might also be relevant to the environmental impacts of climate change. Unlike the temperate *U. mutabilis,* the Antarctic *Ulva* strains grew and released gametes, which developed into gametophytes at 2 °C. Interestingly, characteristic bacterial communities contributed to the adaptation mechanisms of *Ulva* and could facilitate its performance under Antarctic conditions. We isolated and identified *Ulva*-associated

and AGMPF-providing bacteria within the algal growth-promoting polar microbiome. Although those bacteria are not biogeographically unique, they have distinct functional pathways, such as the isolated strain, which is adapted to cold temperatures and releases thallusin to the benefit of *Ulva*. *Maribacter* sp. BPC-D8 and *Sulfitobacter* sp. BPC-C4 are thus drivers of cold adaptation for *Ulva*. The obtained results will serve as a foundation for future in-depth studies that integrate field and experimental work, including comparative genomics of cold and temperate-adapted AGMPF-releasing bacteria. Additionally, emphasis should be placed on the causative functional interactions between bacteria and algal hosts in response to cold or a combination of stressors, which are more prevalent in natural environments than isolated stressors. Exploring the bacterial communities under different (single or combined) stresses will clarify whether an *Ulva*-associated set of core functional traits for adaptation exists and whether the bacterial taxonomic core can be connected to such functional arrangements.

Data Deposition Information

The sequencing reads and assemblies for this whole-genome shotgun project are available in the National Center for Biotechnology Information repository under the BioProject accession number PRJNA828511. The version described in this paper is the first version (NCBI accession number: CP128187). The complete data set of 16S rDNA sequences from the isolated bacteria and the surface microbiome analyses have been deposited at NCBI with the Accession numbers OR957431-OR957479 and SAMN39202330-SAMN39202384 under the same BioProject. Sequence data of the *tufA* gene have the accession numbers PP091296-PP091308. The provided Sequence IDs are identical to those used in this publication.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00344-024-11507-4.

Acknowledgements This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) in the framework of the priority program (SPP 1158) "Antarctic Research with comparative investigations in Arctic ice areas" (424256657, FG, and TW) and of SFB 1127/2 "ChemBioSys" (239748522, TW). The study received financial support from the Ministry for Economics, Sciences and Digital Society of Thuringia (TMWWDG) under the framework of the Landesprogramm ProDigital (DigLeben-5575/10-9). JFU was funded by a doctoral scholarship from the Konrad-Adenauer-Stiftung. MQ was funded by ANPCyT-DNA PICT 2691 and 0501. The project benefited from the network COST Action CA20106 "Tomorrow's wheat of the sea': *Ulva*, a model for an innovative mariculture", supported by COST (European Cooperation in Science and Technology, www.cost.eu). Finally, we thank the Carlini Station crews for their support, logistics, and hospitality. The authors also appreciate the support of the German sampling team: Hans-Peter Grossart, Ulf Karsten, Alexandra Livenets, and Jonas Zimmermann. The authors thank Georg Pohnert (Friedrich-Schiller-Universität Jena) for his great support in Jena and Inka Bartsch (Alfred-Wegener-Institute, Bremerhaven, Germany) for providing us with valuable cultures of the AWI culture collection).

Author Contribution Fatemeh Ghaderiardakani: formal analysis, investigation, methodology, validation, visualization, writing–original draft, and writing–review & editing. Johann F. Ulrich: investigation visualization, and writing—review & editing. Emanuel Barth: formal analysis methodology. Maria Liliana Quartino: investigation, project administration, supervision, and writing—review & editing. Thomas Wichard: conceptualization, data curation, funding acquisition, methodology, project administration, supervision, validation, writing—original draft, and writing—review & editing.

Funding Open Access funding enabled and organized by Projekt DEAL.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Abdul Malik SA, Bedoux G, Garcia Maldonado JQ, Freile-Pelegrín Y, Robledo D, Bourgougnon N (2020) Chapter ten—defence on surface: macroalgae and their surface-associated microbiome. In: Bourgougnon N (ed) Advances in botanical research, vol 95. Academic Press, London, pp 327–368. https://doi.org/10.1016/bs.abr.2019.11.009

Abele D, Vazquez S, Buma AGJ, Hernandez E, Quiroga C, Held C, Frickenhaus S, Harms L, Lopez JL, Helmke E, Mac Cormack WP (2017) Pelagic and benthic communities of the antarctic ecosystem of Potter Cove: genomics and ecological implications. Mar Genom 33:1–11. https://doi.org/10.1016/j.margen.2017.05.001

Alsufyani T, Califano G, Deicke M, Grueneberg J, Weiss A, Engelen AH, Kwantes M, Mohr JF, Ulrich JF, Wichard T (2020) Macroalgal–bacterial interactions: identification and role of thallusin in morphogenesis of the seaweed *Ulva* (Chlorophyta). J Exp Bot 71(11):3340–3349. https://doi.org/10.1093/jxb/eraa066

Alsufyani T, Weiss A, Wichard T (2017) Time course exo-metabolomic profiling in the green marine macroalga *Ulva* (Chlorophyta) for identification of growth phase-dependent biomarkers. Mar Drugs 15(1):14. https://doi.org/10.3390/md15010014

Alvarado P, Huang Y, Wang J, Garrido I, Leiva S (2018) Phylogeny and bioactivity of epiphytic Gram-positive bacteria isolated from three co-occurring antarctic macroalgae. Anton Leeuw 111(9):1543–1555. https://doi.org/10.1007/s10482-018-1044-6

Bischoff-Bäsmann N, Wiencke C (1996) Temperature requirements for growth and survival of Antarctic Rhodophyta. J Phycol 32:25–535. https://doi.org/10.1111/j.0022-3646.1996.00525.x

Blomme J, Wichard T, Jacobs TB, De Clerck O (2023) *Ulva*: an emerging green seaweed model for systems biology. J Phycol 59(3):433–440. https://doi.org/10.1111/jpy.13341

Bowman JP (2017) Genomics of psychrophilic bacteria and archaea. In: Margesin R (ed) Psychrophiles: from biodiversity to biotechnology. Springer, Cham, pp 345–387. https://doi.org/10.1007/978-3-319-57057-0_15

Burke C, Steinberg P, Rusch D, Kjelleberg S, Thomas T (2011) Bacterial community assembly based on functional genes rather than species. Proc Natl Acad Sci USA 108(34):14288–14293. https://doi.org/10.1073/pnas.1101591108

Califano G, Wichard T (2018) Preparation of axenic cultures in *Ulva* (Chlorophyta). In: Reddy C (ed) Protocols for macroalgae research. CRC Press, Boca Raton, pp 159–171

Califano G, Kwantes M, Abreu MH, Costa R, Wichard T (2020) Cultivating the macroalgal holobiont: Effects of integrated multitrophic aquaculture on the microbiome of *Ulva rigida* (Chlorophyta). Front Mar Sci 7:52. https://doi.org/10.3389/fmars.2020.00052

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016) DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods 13(7):581–583. https://doi.org/10.1038/nmeth.3869

Callow JA, Callow ME (2006) Biofilms. In: Fusetani N, Clare AS (eds) Antifouling compounds. Springer, Berlin, pp 141–171

Charrier B, Abreu MH, Araujo R, Bruhn A, Coates JC, De Clerck O, Katsaros C, Robaina RR, Wichard T (2017) Furthering knowledge of seaweed growth and development to facilitate sustainable aquaculture. New Phytol 216(4):967–975. https://doi.org/10.1111/nph.14728

Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH (2020) GTDB-Tk: a toolkit to classify genomes with the genome taxonomy database. Bioinformatics 36(6):1925–1927. https://doi.org/10.1093/bioinformatics/btz848

Chojak-Koźniewska J, Kuźniak E, Zimny J (2018) The effects of combined abiotic and pathogen stress in plants: insights from salinity and *Pseudomonas syringae* pv *lachrymans* interaction in cucumber. Front Plant Sci 9:1691. https://doi.org/10.3389/fpls.2018.01691

Clements CS, Hay ME (2023) Disentangling the impacts of macroalgae on corals via effects on their microbiomes. Front Ecol Evol 11:1083341. https://doi.org/10.3389/fevo.2023.1083341

Cui J, Chen C, Tan H, Huang Y, Chen X, Xin R, Liu J, Huang B, Xie E (2022) Taxonomic delimitation of the monostromatic green algal genera *Monostroma* Thuret 1854 and *Gayralia Vinogradova* 1969 (Ulotrichales, Chlorophyta). Diversity 14(9). https://doi.org/10.3390/d14090773

de Vries J, de Vries S, Curtis BA, Zhou H, Penny S, Feussner K, Pinto DM, Steinert M, Cohen AM, von Schwartzenberg K, Archibald JM (2020) Heat stress response in the closest algal relatives of land plants reveals conserved stress signaling circuits. Plant J 103(3):1025–1048. https://doi.org/10.1111/tpj.14782

Dhiman S, Ulrich JF, Wienecke P, Wichard T, Arndt H-D (2022) Stereoselective total synthesis of (−)-thallusin for bioactivity profiling. Angew Chem Int 61(39):e202206746. https://doi.org/10.1002/anie.202206746

Dittami SM, Duboscq-Bidot L, Perennou M, Gobet A, Corre E, Boyen C, Tonon T (2016) Host–microbe interactions as a driver of acclimation to salinity gradients in brown algal cultures. ISME J 10(1):51–63. https://doi.org/10.1038/ismej.2015.104

Duarte CM, Middelburg JJ, Caraco N (2005) Major role of marine vegetation on the oceanic carbon cycle. Biogeosciences 2(1):1–8. https://doi.org/10.5194/bg-2-1-2005

Egan S, Harder T, Burke C, Steinberg P, Kjelleberg S, Thomas T (2013) The seaweed holobiont: understanding seaweed–bacteria interactions. FEMS Microbiol Rev 37(3):462–476. https://doi.org/10.1111/1574-6976.12011

Eggert A, Wiencke C (2000) Adaptation and acclimation of growth and photosynthesis of five Antarctic red algae to low temperatures. Polar Biol 23(9):609–618. https://doi.org/10.1007/s003000000130

Famà P, Wysor B, Kooistra WHCF, Zuccarello GC (2002) Molecular phylogeny of the genus *Caulerpa* (Caulerpales, chlorophyta) inferred from chloroplast tufa gene. J Phycol 38(5):1040–1050. https://doi.org/10.1046/j.1529-8817.2002.t01-1-01237.x

Føyn B (1958) Über die Sexualität und den Generationswechsel von *Ulva mutabilis*. Arch Protistenk 102:473–480

Frank JA, Reich CI, Sharma S, Weisbaum JS, Wilson BA, Olsen GJ (2008) Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. Appl Environ Microbiol 74(8):2461–2470. https://doi.org/10.1128/aem.02272-07

Friedrich MW (2012) Bacterial communications on macroalgae. In: Wiencke C, Bischof K (eds) Seaweed biology. Springer, Heidelberg

Fromm T, Oberdieck C, Matz T, Wesche C (2020) Expeditions to antarctica: ANT-Land 2019/20 Neumayer Station III, Kohnen Station, Flight Operations and Field Campaigns., vol 745. Reports on Polar and Marine Research. Alfred Wegener Institute for Polar and Marine Research, Bremerhaven

Gaitan-Espitia JD, Schmid M (2020) Diversity and functioning of antarctic seaweed microbiomes. In: Gómez I, Huovinen P (eds) Antarctic seaweeds: diversity, adaptation and ecosystem services. Springer, Cham, pp 279–291. https://doi.org/10.1007/978-3-030-39448-6_14

Galván V, Pascutti F, Sandoval NE, Lanfranconi MP, Lozada M, Araboloza AL, Mac Cormack WP, Alvarez HM, Gramajo HC, Dionisi HM (2023) High wax ester and triacylglycerol biosynthesis potential in coastal sediments of Antarctic and Subantarctic environments. PLoS ONE 18(7):e0288509. https://doi.org/10.1371/journal.pone.0288509

García-López M, Meier-Kolthoff JP, Tindall BJ, Gronow S, Woyke T, Kyrpides NC, Hahnke RL, Göker M (2019) Analysis of 1,000 type-strain genomes improves taxonomic classification of Bacteroidetes. Front Microbiol 10:2083. https://doi.org/10.3389/fmicb.2019.02083

Ghaderiardakani F, Coates JC, Wichard T (2017) Bacteria-induced morphogenesis of *Ulva intestinalis* and *Ulva mutabilis* (Chlorophyta): a contribution to the lottery theory. FEMS Microbiol Ecol 93(8):fix094. https://doi.org/10.1093/femsec/fix094

Ghaderiardakani F, Quartino ML, Wichard T (2020) Microbiome-dependent adaptation of seaweeds under environmental stresses: a perspective. Front Mar Sci 7:575228. https://doi.org/10.3389/fmars.2020.575228

Ghaderiardakani F, Langhans L, Kurbel VB, Fenizia S, Wichard T (2022) Metabolite profiling reveals insights into the species-dependent cold stress response of the green seaweed holobiont *Ulva* (Chlorophyta). Environ Exp Bot 200:104913. https://doi.org/10.1016/j.envexpbot.2022.104913

Grueneberg J, Engelen AH, Costa R, Wichard T (2016) Macroalgal morphogenesis induced by waterborne compounds and bacteria in coastal seawater. PLoS ONE 11(1):e0146307. https://doi.org/10.1371/journal.pone.0146307

Gu K, Liu Y, Jiang T, Cai C, Zhao H, Liu X, He P (2022) Molecular response of *Ulva prolifera* to short-term high light stress revealed by a multi-omics approach. Biology (Basel). https://doi.org/10.3390/biology11111563

Hahnke RL, Bennke CM, Fuchs BM, Mann AJ, Rhiel E, Teeling H, Amann R, Harder J (2015) Dilution cultivation of marine heterotrophic bacteria abundant after a spring phytoplankton bloom in

the North Sea. Environ Microbiol 17(10):3515–3526. https://doi.org/10.1111/1462-2920.12479
Hernández EA, Lopez JL, Piquet AMT, Mac Cormack WP, Buma AG (2019) Changes in salinity and temperature drive marine bacterial communities' structure at Potter Cove, Antarctica. Polar Biol 42:2177–2191. https://doi.org/10.1007/s00300-019-02590-5
Hmani I, Ghaderiardakani F, Ktari L, Bour ME, Wichard T (2024) High-temperature stress induces bacteria-specific adverse and reversible effects on *Ulva* Chlorophyta growth and its chemosphere in a reductionist model system. Bot Mar 67(2):131–138. https://doi.org/10.1515/bot-2023-0053
Ilicic D, Ionescu D, Woodhouse J, Grossart H-P (2023) Temperature-related short-term succession events of bacterial phylotypes in Potter Cove, Antarctica. Genes 14(5):1051
Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S (2018) High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun 9(1):5114. https://doi.org/10.1038/s41467-018-07641-9
Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. Oikos 69(3):373–386. https://doi.org/10.2307/3545850
Juul L, Nissen SH, Bruhn A, Alexi N, Jensen SK, Hammershøj M, Dalsgaard TK (2024) Ulva species: a critical review on the green seaweed as a source of food protein. Trends Food Sci Technol 149:104534. https://doi.org/10.1016/j.tifs.2024.104534
Kessler RW, Weiss A, Kuegler S, Hermes C, Wichard T (2018) Macroalgal–bacterial interactions: role of dimethylsulfoniopropionate in microbial gardening by *Ulva* (Chlorophyta). Mol Ecol 27(8):1808–1819. https://doi.org/10.1111/mec.14472
Krucon T, Dziewit L, Drewniak L (2021) Insight into ecology, metabolic potential, and the taxonomic composition of bacterial communities in the periodic water pond on King George Island (Antarctica). Front Microbiol. https://doi.org/10.3389/fmicb.2021.708607
Kwantes M, Wichard T (2022) The APAF1_C/WD40 repeat domain-encoding gene from the sea lettuce *Ulva mutabilis* sheds light on the evolution of NB-ARC domain-containing proteins in green plants. Planta 255(4):76. https://doi.org/10.1007/s00425-022-03851-0
Lu DC, Wang FQ, Amann RI, Teeling H, Du ZJ (2023) Epiphytic common core bacteria in the microbiomes of co-located green (*Ulva*), brown (*Saccharina*) and red (*Grateloupia*, *Gelidium*) macroalgae. Microbiome 11:126. https://doi.org/10.1186/s40168-023-01559-1
Mantri VA, Kazi MA, Balar NB, Gupta V, Gajaria T (2020) Concise review of green algal genus *Ulva* Linnaeus. J Appl Phycol 32(5):2725–2741. https://doi.org/10.1007/s10811-020-02148-7
Marshall K, Joint I, Callow ME, Callow JA (2006) Effect of marine bacterial isolates on the growth and morphology of axenic plantlets of the green alga *Ulva linza*. Micro Ecol 52(2):302–310. https://doi.org/10.1007/s00248-006-9060-x
Matsuo Y, Suzuki M, Kasai H, Shizuri Y, Harayama S (2003) Isolation and phylogenetic characterization of bacteria capable of inducing differentiation in the green alga *Monostroma oxyspermum*. Environ Microbiol 5(1):25–35. https://doi.org/10.1046/j.1462-2920.2003.00382.x
Matsuo Y, Imagawa H, Nishizawa M, Shizuri Y (2005) Isolation of an algal morphogenesis inducer from a marine bacterium. Science 307:1598. https://doi.org/10.1126/science.1105486
Nedashkovskaya OI, Kim SB, Lee KH, Bae KS, Frolova GM, Mikhailov VV, Kim IS (2005) *Pibocella ponti* gen. nov., sp. nov., a novel marine bacterium of the family Flavobacteriaceae isolated from the green alga *Acrosiphonia sonderi*. Int J Syst Evol Microbiol 55(Pt 1):177–181. https://doi.org/10.1099/ijs.0.63251-0
Nguyen D, Ovadia O, Guttman L (2023) Temporal force governs the microbial assembly associated with *Ulva fasciata* (Chlorophyta) from an integrated multi-trophic aquaculture system. Front Microbiol 14:1223204. https://doi.org/10.3389/fmicb.2023.1223204
Quartino ML, Zaixso HE, de Zaixso ALB (2005) Biological and environmental characterization of marine macroalgal assemblages in Potter Cove, South Shetland Islands, Antarctica. Bot Mar 48(3):187–197. https://doi.org/10.1515/bot2005.029
Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41(D1):D590–D596. https://doi.org/10.1093/nar/gks1219
Risely A (2020) Applying the core microbiome to understand host–microbe systems. J Anim Ecol 89(7):1549–1558. https://doi.org/10.1111/1365-2656.13229
Saunders GW, Kucera H (2010) An evaluation of *rbcL*, *tufA*, UPA, LSU and ITS as DNA barcode markers for the marine green macroalgae. Cryptogamie Algologie 2010(4):487–528
Saha M, Dittam, SM, Chan CX, Raina J-B, Stock W, Ghaderiardakani F, Valathuparambil Baby John AM, Corr S, Schleyer G, Todd J, Cardini U, Bengtsson MM, Prado S, Skillings D, Sonnenschein EC, Engelen AH, Wang G, Wichard T, Brodie J, Leblanc C, Egan S (2024) Progress and future directions for seaweed holobiont research. New Phytol, 244: 364-376. https://doi.org/10.1111/nph.20018
Schmieder R, Edwards R (2011) Quality control and preprocessing of metagenomic datasets. Bioinformatics 27(6):863–864. https://doi.org/10.1093/bioinformatics/btr026
Seymour J, Amin S, Raina J-B, Stocker R (2017) Zooming in on the phycosphere: the ecological interface for phytoplankton-bacteria relationships. Nat Microbiol. https://doi.org/10.1038/nmicrobiol.2017.65
Shade A, Handelsman J (2012) Beyond the Venn diagram: the hunt for a core microbiome. Environ Microbiol 14(1):4–12. https://doi.org/10.1111/j.1462-2920.2011.02585.x
Simon C, McHale M, Sulpice R (2022) Applications of *Ulva* biomass and strategies to improve its yield and composition: a perspective for *Ulva* aquaculture. Biology (Basel). https://doi.org/10.3390/biology11111593
Spoerner M, Wichard T, Bachhuber T, Stratmann J, Oertel W (2012) Growth and thallus morphogenesis of *Ulva mutabilis* (Chlorophyta) depends on a combination of two bacterial species excreting regulatory factors. J Phycol 48(6):1433–1447. https://doi.org/10.1111/j.1529-8817.2012.01231.x
Steinhagen S, Barco A, Wichard T, Weinberger F (2019) Conspecificity of the model organism *Ulva mutabilis* and *Ulva compressa* (Ulvophyceae, Chlorophyta). J Phycol 55(1):25–36. https://doi.org/10.1111/jpy.12804
Stratmann J, Paputsoglu G, Oertel W (1996) Differentiation of *Ulva mutabilis* (Chlorophyta) gametangia and gamete release are controlled by extracellular inhibitors. J Phycol 32(6):1009–1021. https://doi.org/10.1111/j.0022-3646.1996.01009.x
Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739. https://doi.org/10.1093/molbev/msr121
Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI (2007) The human microbiome project. Nature 449:804–810. https://doi.org/10.1038/nature06244.
Ulrich JF, Gräfe MS, Dhiman S, Wienecke P, Arndt H-D, Wichard T (2022) Thallusin quantification in marine bacteria and algae cultures. Mar Drugs 20(11):690. https://doi.org/10.3390/md20110690
van der Loos LM, Eriksson BK, Falcão Salles J (2019) The macroalgal holobiont in a changing sea. Trends Microbiol 27:635–650. https://doi.org/10.1016/j.tim.2019.03.002

van der Loos LM, D'Hondt S, Engelen AH, Pavia H, Toth GB, Willems A, Weinberger F, De Clerck O, Steinhagen S (2022) Salinity and host drive *Ulva*-associated bacterial communities across the Atlantic-Baltic Sea gradient. Mol Ecol. https://doi.org/10.1111/mec.16462

Vila E, Hornero-Méndez D, Azziz G, Lareo C, Saravia V (2019) Carotenoids from heterotrophic bacteria isolated from Fildes Peninsula, King George Island, Antarctica. Biotechnol Rep 21:e00306. https://doi.org/10.1016/j.btre.2019.e00306

Wahl M, Goecke F, Labes A, Dobretsov S, Weinberger F (2012) The second skin: ecological role of epibiotic biofilms on marine organisms. Front Microbiol 3:292. https://doi.org/10.3389/fmicb.2012.00292

Weiss A, Costa R, Wichard T (2017) Morphogenesis of *Ulva mutabilis* (Chlorophyta) induced by *Maribacter* species (Bacteroidetes, Flavobacteriaceae). Bot Mar 60(2):197–206. https://doi.org/10.1515/bot-2016-0083

Wichard T (2015) Exploring bacteria-induced growth and morphogenesis in the green macroalga order Ulvales (Chlorophyta). Front Plant Sci 6:86. https://doi.org/10.3389/fpls.2015.00086

Wichard T (2023) From model organism to application: bacteria-induced growth and development of the green seaweed *Ulva* and the potential of microbe leveraging in algal aquaculture. Semin Cell Dev Biol 134:69–78. https://doi.org/10.1016/j.semcdb.2022.04.007

Wichard T, Charrier B, Mineur F, Bothwell JH, Clerck OD, Coates JC (2015) The green seaweed *Ulva*: a model system to study morphogenesis. Front Plant Sci 6:72. https://doi.org/10.3389/fpls.2015.00072

Wiencke C, Clayton MN (2002) Antarctic seaweeds. Synopses of the Antarctic benthos, vol 9. Gantner Verlag KG. A.R.G, Ruggell

Wiencke C, Dieck IT (1990) Temperature requirements for growth and survival of macroalgae from Antarctica and Southern Chile. Mar Ecol Prog Ser 59(1–2):157–170. https://doi.org/10.3354/meps059157

Wiencke C, Ferreyra G, Arntz W, Rinaldi C (1998) The Potter Cove coastal ecosystem, Antarctica. Ber Polarforsch 299

Wiencke C, Luder UH, Roleda MY (2007) Impact of ultraviolet radiation on physiology and development of zoospores of the brown alga *Alaria esculenta* from Spitsbergen. Physiol Plant 130(4):601–612. https://doi.org/10.1111/j.1399-3054.2007.00923.x

William S, Feil H, Copeland A (2004) Bacterial DNA isolation CTAB protocol bacterial genomic DNA isolation using CTAB materials & reagents. DOE Joint Genome Institute, Berkeley, CA, USA, p 4

Zhao X, Tang X, Zhang H, Qu T, Wang Y (2016) Photosynthetic adaptation strategy of *Ulva prolifera* floating on the sea surface to environmental changes. Plant Physiol Biochem 107:116–125. https://doi.org/10.1016/j.plaphy.2016.05.036

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.