



# Manipulation of the surface-associated bacteria on *Asparagopsis taxiformis* and its effect on seaweed growth and halogenated natural products

S. Blanco<sup>1</sup> · A. H. Campbell<sup>1,2</sup> · S. F. Cummins<sup>1</sup> · D. A. Heyne<sup>1</sup> · N. A. Paul<sup>1</sup>

Received: 17 January 2025 / Revised: 11 April 2025 / Accepted: 22 April 2025 / Published online: 14 May 2025  
© The Author(s) 2025

## Abstract

Epiphytic bacteria can play a crucial role in regulating seaweed traits, yet their specific contributions to both host growth and natural products remain unclear for most seaweeds. Here, we explored the effects of manipulating the surface-associated bacteria on the growth and halogenated compounds of two domesticated strains of *Asparagopsis taxiformis* sporophytes under intensive culture. Under standard conditions, sporophytes had an average surface bacterial density of 1 per 32  $\mu\text{m}^2$ . Following five different antibiotic treatments at 10 mg L<sup>-1</sup> for 24 h (i.e., penicillin, streptomycin, kanamycin, gentamicin, and vancomycin), bacterial densities were significantly reduced depending on the antibiotic, averaging 1 per 90  $\mu\text{m}^2$ . Antibiotics treatment led to 22–89% reductions in bacterial density compared to controls after 2 weeks, with gentamicin and streptomycin most effective (86–89% reductions to ~1 per 260  $\mu\text{m}^2$ ), followed by vancomycin (77%), kanamycin (23%) and penicillin (22%). We examined the impacts on growth and the concentration of two major halogenated compounds, bromoform and dibromoacetic acid, with a combined concentration of 1–2% dry weight. Our results highlight that the antibiotic treatments with the lowest bacterial densities (gentamicin and streptomycin) had higher growth rates than the control (> 50% increase). We observed a negative impact on the bromoform and dibromoacetic acid concentrations, with 42% and 35% reductions, respectively, dependent on strain and antibiotic. Overall, our study demonstrates that the epiphytic bacteria in *A. taxiformis* cultures can be manipulated to enhance seaweed biomass production, but there may be a trade off in natural product levels in some culture lines.

**Keywords** *Asparagopsis* · Rhodophyta · Microbiome · Bromoform · Antibiotic

## Introduction

The red seaweed genus *Asparagopsis* (Bonnemaisoniaceae) is known for producing a wide array of over 100 halogenated compounds, including haloforms, haloacids and haloketones (McConnell and Fenical 1977; Woolard et al. 1979). These compounds have industrial applications (Machado et al. 2014; Thépot et al. 2022), but are also thought to play roles in the seaweed defence mechanisms against bacteria

(Paul et al. 2006). The microbiome, which consists of bacteria, fungi, archaea, and other microorganisms, has been shown to regulate key host functions like development and stress response (Singh and Reddy 2014; Ghaderiardakani et al. 2020). Bacteria are the dominant group of epiphytic microbes, due to their ability to rapidly colonise and metabolise seaweed exudates (Lachnit et al. 2009). Therefore, disrupting or modifying the microbiome could impact host traits, in either positive or negative ways (Marshall et al. 2006; Tapia et al. 2016). Much of the work to date on seaweed has explored the indirect effects of environmental pressure (e.g., temperature) and pathogenic bacteria on seaweeds (Ward et al. 2020; Spagnuolo and Genovese 2024), with little information on the positive impact of bacteria on seaweed aquaculture to draw upon.

In both agriculture and aquaculture, manipulation of the microbiota has shown positive results for production, such as improved disease resistance and growth in plants and

✉ S. Blanco  
silvia.blanco@research.usc.edu.au

<sup>1</sup> School of Science, Technology and Engineering, University of the Sunshine Coast, Maroochydore DC, QLD 4558, Australia

<sup>2</sup> School of Health, University of the Sunshine Coast, Maroochydore DC, QLD 4558, Australia

fish (Montalban-Arques et al. 2015; Brugman et al. 2018). In other marine organisms, such as corals, the introduction of microorganisms can reprogram genetic and metabolic pathways related to immune function and stress resistance, leading to enhanced health and resilience to bleaching (Peixoto et al. 2017; Rosado et al. 2019). While such techniques have not yet been applied to seaweed cultivation, there is growing interest in this approach driven by a desire to boost production in aquaculture systems. The review by Li et al. (2023) outlines a pathway for improving seaweed aquaculture through targeted microbiota manipulation, highlighting how understanding and managing microbial communities could enhance seaweed productivity, resilience, and commercial viability. This is particularly relevant for *Asparagopsis*, which offers a promising solution to combat global warming by reducing the production and release of methane in ruminant animals like cows, and sheep (Machado et al. 2016; Li et al. 2018; Roque et al. 2021). Methane is a powerful greenhouse gas and research has shown that when ruminants are fed *Asparagopsis*, certain compounds, especially bromoform, can significantly lower methane production during fermentation, helping to reduce global warming by mitigating the enteric methane production and emission in ruminant animals. Therefore there is a need to explore the link between growth and microbial interactions in this newly emerging commercial algal species. This would support global efforts to intensify and scale production for methane reduction (Abbott et al. 2020). Environmental research on a related species *Delisea pulchra* (Bonnemaisoniaceae), has shown that antibiotic disruption of the microbiome can cause temporary alterations, but the microbiome often reverts to its original state, sometimes leading to bleaching diseases (Longford et al. 2019). Questions therefore remain whether manipulating the microbiome of cultured *Asparagopsis* can alter traits such as growth, morphology, and synthesis of natural products. Furthermore, the land-based culture setting is different to nature, and correspondingly the microbiome of cultured *Asparagopsis* strains can be quite different to that of the wild type (Zhao et al. 2022). In addition, moving seaweeds from wild to cultured environments has been accompanied by reduced growth and performance (Zozaya-Valdés et al. 2016), raising the question of whether microbiome management is also important for domestication, and potentially long-term success of intensive cultures.

Our study specifically asks: (1) Can we disrupt the surface-associated bacteria on *A. taxiformis* using antibiotics? and (2) How do these different antibiotics treatments affect the overall performance of *A. taxiformis* in terms of growth and halogenated compound production? To investigate this, we used five different antibiotics (kanamycin, streptomycin, gentamicin, penicillin, vancomycin, and a combination of all) to manipulate the density of epiphytic bacteria on two domesticated strains of *A. taxiformis* sporophytes that

had been in intensive culture for 2–3 years. In this study we describe, for the first time, the natural product chemistry of *A. taxiformis* from Queensland, Australia (Lineage 6, Zhao et al. (2022)), and then focus specifically on the two key metabolites by quantity, bromoform and dibromoacetic acid (DBAA).

## Materials & methods

### Study organism

*Asparagopsis taxiformis* (Delile) Trevisan de Saint-Léon has a heteromorphic life history, alternating between a filamentous tetrasporophyte (diploid) and an erect, plumose gametophyte (haploid). Sporophytes of *A. taxiformis* (Lineage 6, hereafter *Asparagopsis*) were collected in 2018 and 2019 from Moffatt Beach, Australia (26.7934°S, 153.1355°E), and kept in culture at the University of the Sunshine Coast under the following cultivation conditions: 0.5 g L<sup>-1</sup>, 22 °C, under 30 μmol photons m<sup>-2</sup> s<sup>-1</sup> blue:white light with a 16:8 h light:dark photoperiod. The cultivation medium is changed weekly with autoclaved seawater enriched with half-strength f/2 nutrients (adapted from Guillard and Ryther (1962)). The enrichment solution contains vitamins B<sub>1</sub> (thiamine), B<sub>7</sub> (biotin) and B<sub>12</sub> (cobalamin) that could mask the inability of seaweed to produce vitamins and potential provisioning by bacteria (for example, if *Asparagopsis* is auxotrophic for these vitamins, i.e., unable to synthesise them). GeO<sub>2</sub> at 0.4 mg L<sup>-1</sup> was added to control diatom growth (Lewin 1966). The following experiments were conducted in 2023, or approximately 2–3 years since domestication of the 2018 Strain and 2019 Strain (hereafter S-2018 and S-2019).

### Antibiotic treatment

To assess the effect of bacterial disruption on *Asparagopsis*, antibiotics with different modes of action were selected (Table 1). Aminoglycoside antibiotics such as kanamycin, streptomycin, and gentamicin (derived from Actinomycetes) are effective against a broad spectrum of Gram-negative bacteria, and a few Gram-positive bacteria. In contrast, penicillin targets mainly Gram-positive, and some Gram-negative, whereas vancomycin is ineffective against Gram-negative bacteria.

The treatments used in this study drew upon antibiotic treatments from different studies to manipulate the microbiome in fish and seaweeds (Klein Jan et al. 2017; Longford et al. 2019; Gao et al. 2021; Xavier et al. 2024). Antibiotic treatments were done using *Asparagopsis* sporophyte cultures in 50 mL polypropylene jars at an initial stock density of 0.5 g L<sup>-1</sup> (0.025 g of spun-dried biomass per jar). Treatments with both strains, S-2018 and S-2019, were run concurrently (i.e.

**Table 1** Antibiotics used and their mode of action and spectrum activity

| Antibiotic                  | Mode of action                                                                                                                                            | Spectrum                                                 | References                 |
|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------|----------------------------|
| Kanamycin sulfate           | Aminoglycoside—Binds to the 30 s ribosomal subunit causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth | Broad spectrum; effective against Gram – /Gram +         | Papich (2015)              |
| Streptomycin                | Aminoglycoside – Binds to the s12 protein of the 30 s ribosomal subunit inhibiting protein synthesis                                                      | Broad spectrum; most Gram – and a few Gram +             | Vardanyan and Hruby (2006) |
| Gentamicin                  | Aminoglycoside—Inhibits protein synthesis by binding to the 30 s subunit of the ribosome                                                                  | Broad spectrum; effective against Gram – and some Gram + | Reeves et al. (2016)       |
| Penicillin G sodium sulfate | Inhibition of the cell wall synthesis through binding proteins (PBPs), inhibiting peptidoglycan chain cross-linking                                       | Narrow spectrum; targeting Gram +                        | Hruby and Schiel (1996)    |
| Vancomycin                  | Blocks bacterial cell wall biosynthesis at the level of peptidoglycan biosynthesis                                                                        | Narrow spectrum; Gram +                                  | McLawhon (2012)            |

‘Strain’ was a fixed factor). Before the antibiotic treatments, biomass of both strains was held under standard conditions for a two-week adaptation period. Antibiotic treatments were formulated in autoclaved seawater at a concentration of 10 mg L<sup>-1</sup> for each of the five antibiotics: kanamycin, streptomycin, gentamicin, penicillin, and vancomycin (Sigma-Aldrich, USA). Additionally, a mixed treatment with all five antibiotics was included with a total antibiotic concentration of 50 mg L<sup>-1</sup>. The biomass was evenly mixed and randomly assigned to the antibiotic treatments (n = 5 jars per antibiotic treatment). The biomass was exposed to antibiotics for 24 h then rinsed thoroughly using autoclaved seawater for 3 min. Each replicate jar was then returned to standard cultivation conditions for two weeks. The water was exchanged one-week post-antibiotic treatment and then on day 14 the seaweed was harvested to measure the growth and bacterial densities. Portions of the biomass were snap-frozen and stored at –80°C until analysis of natural products.

## Bacterial density

The bacterial density of *Asparagopsis* surface-associated bacteria was quantified by fluorescence microscopy using Syto 9 Green Fluorescent Nucleic Acid Stain (Ex/Em: RNA 486/501 nm; DNA 485/498 nm) (Invitrogen, USA) using the following steps based on a preliminary trial. For each replicate (n = 5 cultures), three subsamples (n = 3) were taken, and fixed in a 4% formaldehyde solution made in filtered autoclaved seaweed (0.22 µm). The filaments were stained in the dark for 30 min with 5 µL of a 25 µM solution of Syto 9 stain solution from an initial stock solution prepared in EDTA (pH 8, 10 mM). Following staining, the filaments were rinsed three times in filtered autoclaved seawater before being examined under a fluorescence microscope

equipped with a GFP filter (Leica DM 5500 B, coupled with a DMC6200 camera; Germany). To minimize the impact of seaweed epifluorescence, three individual sections of filament from each subsample were randomly selected for bacterial density measurement. The sampling areas were kept at least 25 µm away from the tips (new growth) and branching locations.

A region of interest of 1024 × 1024 pixels was first established at 200 × magnification and from each region bacteria were quantified from an image taken at 400 × magnification. Three images were captured from random locations within each sample. Each image comprised a compilation of 10 shots obtained using the z-stack tool in the Leica LAS X software. The images were processed using the ImageJ program (Schneider et al. 2012). A randomized grid of 500 µm<sup>2</sup> was applied to each image, and bacteria were counted in three squares, with the results averaged as bacteria per square micrometer (bacteria µm<sup>-2</sup>).

## Growth

To measure the growth rate, the *Asparagopsis* biomass was spun-dried using calico bags (Koh-I-Noor, Czech Republic) and weighed on an analytical scale to 3 decimal places (Ohaus, USA). The specific growth rate (SGR) was calculated for each replicate using the following equation:

$$SGR(\% \text{ day}^{-1}) = \left( \frac{\ln B_f - \ln B_i}{T} \right) \times 100$$

where  $B_f$  is the final biomass (measured on day 14 after antibiotic treatment) and  $B_i$  is the initial biomass (0.5 mg, day 1) following antibiotic treatment, and  $T$  is the number of days in culture (14 days). At the end of the experiment,

most of the biomass was snap-frozen and placed at  $-80^{\circ}\text{C}$  for natural product analysis ('Chemistry', below), and the rest for assessing the bacterial density (above).

## Chemistry

The snap frozen *Asparagopsis* biomass was freeze-dried before the extraction and quantification of natural products. While over 100 halogenated natural products have been identified in the genus *Asparagopsis*, only a limited number are present in quantities above detection levels for gas chromatography-mass spectrometry (GC–MS) and have commercially available standards for quantification. The biomass was freeze-dried for 40 h (Thermo Savant, USA) and milled by adding two tungsten carbide balls ( $D = 5\text{ mm}$ ), and vortexing for 40 s at maximum speed. For extraction, 4 mg milled biomass was combined with 0.350 mL methanol containing naphthalene as the internal standard at a concentration of  $30.1\text{ }\mu\text{g mL}^{-1}$ . The samples were left at  $5^{\circ}\text{C}$  for at least 72 h to ensure esterification before being transferred via glass pipette into GC–MS vials.

All GC–MS analyses were performed on a Perkin Elmer Clarus 580 GC coupled with a Perkin Elmer Clarus SQ8S MS, following the methodology described by Paul et al. (2006). The column used was an Elite-5MS ( $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$ ), with helium as the carrier gas at a constant flow rate of  $1.0\text{ mL min}^{-1}$ . The injection port was set to  $250^{\circ}\text{C}$  with a split ratio of 50:1. The temperature program was operated from  $40^{\circ}\text{C}$  for 1 min, ramping at  $20^{\circ}\text{C min}^{-1}$  until  $250^{\circ}\text{C}$  and holding for 0 min. The mass spectrometer analysed a mass range from 50 to 340 ( $m/z$ ), from 3 to 12 min at 70 eV. Data analysis was conducted using TurboMass software, referencing the National Institute of Standards and Technology internal libraries (2008) for tentative compound identification. Peak areas were determined from the total ion chromatogram. Following the sample injections, a standard curve was generated for each target compound. The concentration of each target compound in the samples was calculated based on the ratio of the target compound's peak area to that of the internal standard, which was then converted to concentration using standard curves. All halogenated compounds are expressed as a percentage of dry matter (% dry matter) with haloacids converted to the original non-esterified form. Commercial standards for dibromochloromethane, bromoform, bromochloroacetic acid (BCAA), dibromoacetic acid (DBAA) and carbon tetrabromide (CT) were sourced from Merck (Sigma Aldrich, USA).

## Statistical analysis

Statistical analyses were performed using R Statistical Software (v4.1.2; R CoreTeam (2021)). The effects of Strain (Fixed factor, two levels: S-2019 and S-2018) and antibiotic

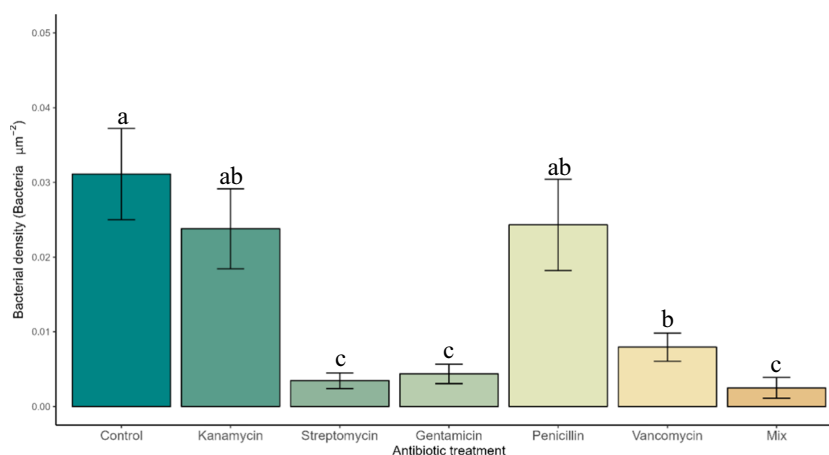
treatment (fixed factor, seven levels including control and mixed treatment) on specific growth rate (SGR), bacterial density, and the concentration of each compound were each assessed using two-factor analysis of variance (ANOVA). Before performing ANOVAs, the assumptions of normality and homogeneity of variances were assessed, with no data transformations required. Where a significant difference for a main effect was found ( $\alpha = 0.05$ ), Tukey's Honest Significant Difference (HSD) multiple comparison was used to determine pairwise differences among treatments. Additionally, variance component analysis was carried out for bacterial density for all treatments and both strains to quantify the proportion of variance attributable to different levels within the hierarchical sampling design. This allowed for a better understanding of differences in bacterial densities within samples (replicate jar [independent culture vessel]  $\rightarrow$  subsamples [individual filament]  $\rightarrow$  locations [within filament – separated by 100–1000  $\mu\text{m}$ ]  $\rightarrow$  square [separated by 10–100  $\mu\text{m}$ ]).

## Results

### Bacterial densities

There were significant differences in the efficacy of the antibiotic treatments to reduce bacterial density, with a substantial 92% reduction from the control (0.031 to 0.002 bacteria  $\mu\text{m}^{-2}$  ( $F_{6,54} = 9.18$ ,  $p < 0.001$ ). There was no overall difference in bacterial densities between the two seaweed strains ( $F_{1,54} = 0.84$ ,  $p = 0.364$ ), nor was there any interaction between strain and antibiotic treatment ( $F_{6,54} = 1.56$ ,  $p = 0.177$ ). These results highlight the different efficacies of antibiotics in reducing bacterial density on *Asparagopsis*. Gentamicin, and streptomycin treatments at  $10\text{ mg L}^{-1}$  resulted in the lowest bacterial densities with an 89% reduction compared to the controls (Tukey's HSD,  $p < 0.05$ ; Fig. 1). Gentamicin resulted in a density of 0.004 bacteria  $\mu\text{m}^{-2}$ , and streptomycin was 0.003 bacteria  $\mu\text{m}^{-2}$ . Vancomycin was less effective but still achieved a 77% reduction in bacterial density. Penicillin and kanamycin treatments did not significantly reduce bacterial densities ( $\sim 0.023$  bacteria  $\mu\text{m}^{-2}$ ). The only other antibiotic treatment that led to a significant reduction in density was the mixed treatment (all antibiotics at the same concentration of the individual treatments mixed treatment, administered at a dosage 4-times higher ( $50\text{ mg L}^{-1}$ ), which was the same as gentamicin and streptomycin). There was no clear relationship between the efficacy of the purported broad-spectrum antibiotics (streptomycin, gentamicin, kanamycin) and those targeting gram-positive bacteria (penicillin and vancomycin).

While there were no overall differences in bacterial densities between the strains, there was significant variability at the smaller scale among and across filaments within intensive



**Fig. 1** Effect of different antibiotics on bacterial density (bacteria  $\mu\text{m}^{-2}$ ) on the surface of *Asparagopsis taxiformis*. Data are presented as mean  $\pm$  SE, pooled across two strains ( $n = 14$ ). Shared letters indicate no significant difference (Tukey's HSD). Broad-spectrum antibiotics are shown in green and those targeted against Gram-positive

bacteria in yellow (each individual treatment is  $10 \text{ mg L}^{-1}$ ). The mix (orange) represents a combination of all antibiotics (to a total of  $50 \text{ mg L}^{-1}$ ). Bacterial counts were made at the end of the growth trial (14-day culture period after a 24-h antibiotic treatment)

cultures (Fig. 2). Focusing on the control cultures, which had the highest bacterial densities, S-2018 displayed a 32% variance between squares ( $10\text{--}100 \mu\text{m}$ ) (Fig. 2, left), the lowest sampling level, whereas the S-2019 showed a 5% variance within squares. This was illustrated by comparing different sampling 'locations' (separated by  $\sim 100\text{--}1000 \mu\text{m}$ ), and corresponding regions of interest ( $1024 \times 2014$  pixels, or  $10\text{--}50 \mu\text{m}$  separation) observed under  $200 \times$  magnification, in the dense biofilm on one part of the surface of the seaweed (Fig. 2A, indicated by an arrow). A variance component analysis (VCA) plot and data by treatment are included in the supplementary section (Fig. S1). The Syto 9 Green Fluorescent nucleic acid stain, which stains both live and dead bacteria, revealed no evidence of bacteria within the tissue (intracellular) of *A. taxiformis*. However, the nuclei of *A. taxiformis* cells were visible (Fig. 2B, arrow), indicating that the bacteria were external to the algal tissue. Some regions of the filaments had higher bacterial density in the selected area (Figs. 2C, D; C includes the square grid [ $22.36 \mu\text{m} \times 22.36 \mu\text{m} = 500 \mu\text{m}^2$ ] used for counting bacteria), whereas other whole areas exhibited low or complete absence of bacteria (Fig. 2E). Higher bacterial densities were also found near the branching points of the filaments (Fig. 2F).

Overall, the variance in bacterial density was primarily driven by the "location" component across most antibiotic treatments, with "replicate" and "square" also contributing (Supplementary Table S1). The location level accounted for the highest percentage of variance in both strains, particularly the kanamycin treatment for S-2019, where location explained 79.18% of the variance. Similarly, in the vancomycin treatment, location explained 79.60% and 64.27% of the variance for S-2018 and S-2019, respectively. These results

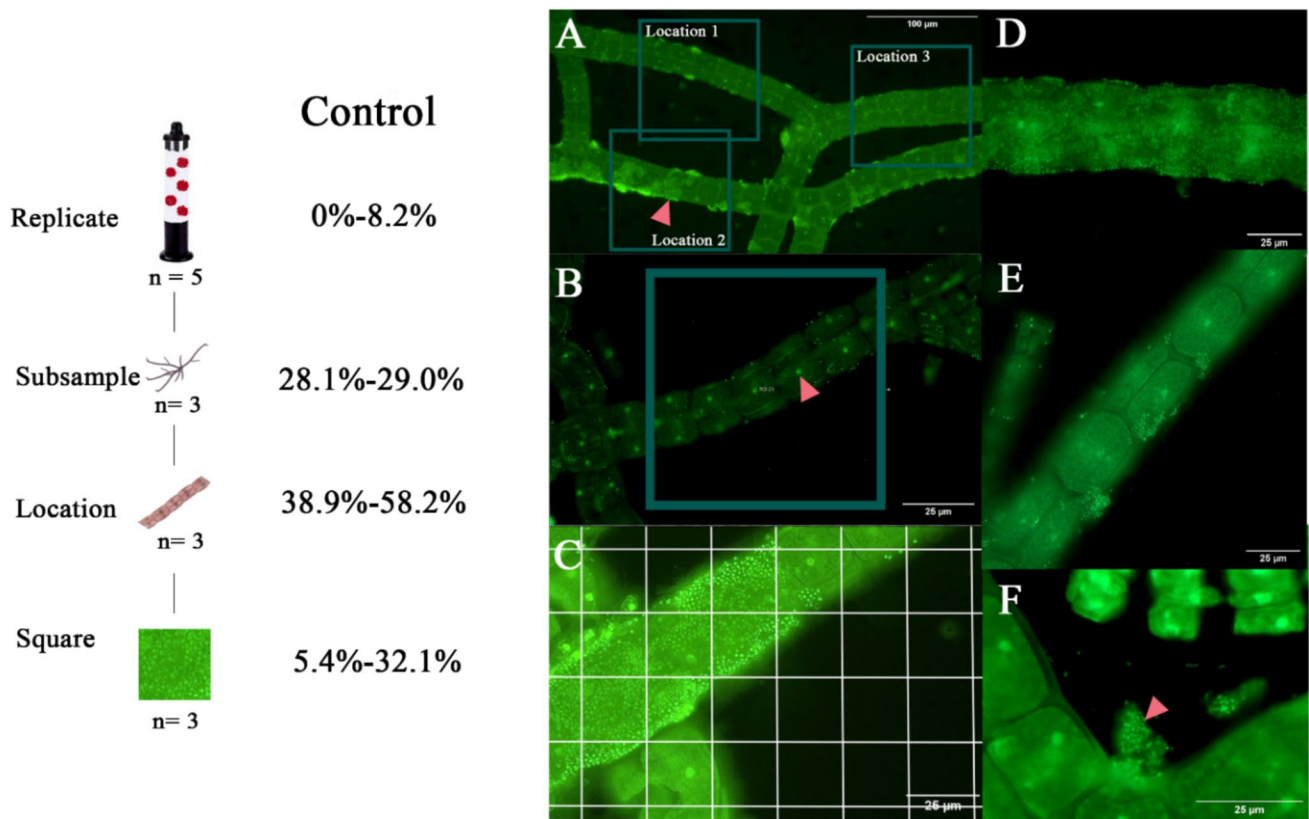
quantify the variability in bacterial density across antibiotic treatments and the control.

### Specific growth rate

There was a significant effect of antibiotic treatment on the growth of *Asparagopsis* ( $F_{6,54} = 2.33$ ,  $p = 0.049$ ), but no effect of strain ( $F_{1,54} = 2.12$ ,  $p = 0.151$ ), nor any interaction between the factors ( $F_{6,54} = 0.77$ ,  $p = 0.590$ ) (Fig. 3). The streptomycin (7.81% growth per day) and gentamicin (7.93%) demonstrated the two highest growth rates, whereas the control and mixed showed the two lowest. Pairwise comparisons (using the conservative Tukey's HSD with 7 pairwise comparisons), highlighted that the streptomycin compared to control ( $p = 0.060$ ) and gentamicin to control ( $p = 0.051$ ), drove significant experiment wide effect of antibiotics in the ANOVA, contributing to the 75–82% increase in growth rate of these treatments compared to the control growth rate. The pooled average specific growth rates were  $6.14\% \pm 0.43 \text{ SE}$  and  $5.43\% \pm 0.29 \text{ SE}$  for S-2018 and S-2019 respectively.

### Chemistry

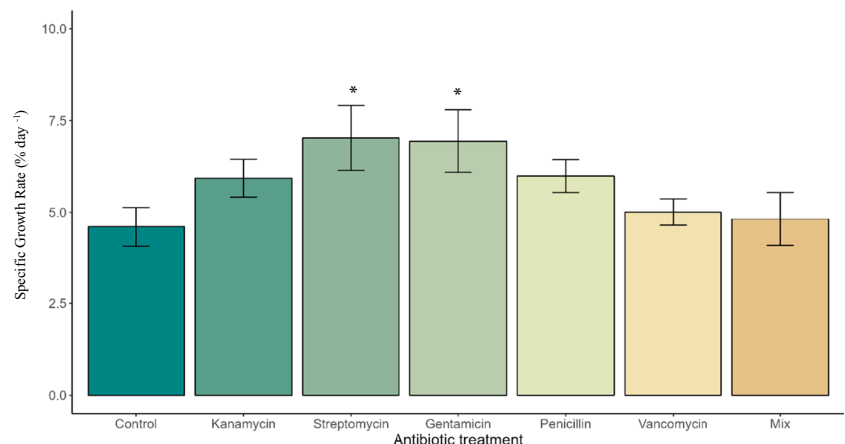
GC–MS analyses consistently identified eight halogenated natural products in the experimental cultures of *Asparagopsis* (Table 2). These included halomethanes (dibromochloromethane, bromoform, carbon tetrabromide), haloacids (DBAA, bromochloroacetic acid, 3,3,-dibromoprop-2-enoic acid) and haloacetones (1,1,3-tribromopropan-2-one and 1,1,3-tribromopropan-2-one). The two major compounds by concentration were bromoform and DBAA, and in general,



**Fig. 2** Fluorescence micrographs of the surface of sporophyte of *Asparagopsis taxiformis* stained with Syto 9 Green fluorescent nucleic acid stain. On the left side of the figure, the variance in density (% total) across different sampling levels are presented (as a range across S-2018 and S-2019), starting from the replicate (hatcher) for the control to subsample (sporophyte), location (filament), and square (within filament). **A–F** Seaweed stained with Syto 9 Green highlighting examples of the surface bacteria. (**A–C**) Illustrate the hierarchical method used to count the bacterial loads on the host surface: **A** Subsample (whole filament) with three ‘locations’ selected

(200  $\times$  magnification; arrow indicating biofilm formation on the filament); **B** A single ‘location’, with the arrow pointing to the *A. taxiformis* nucleus (200  $\times$  magnification); **C** Area used for quantification in ImageJ, where a grid is randomly applied, and counts are taken from three random ‘squares’; **D–F** Highlight observed features of the epiphytic bacteria including a region of the filament densely covered by bacteria (**D**), another area with few bacteria concentrated in small patches (**E**), and branching section of the filament which were excluded from sampling to focus on unbranched sections – arrow pointing to bacterial clumps in a branching section (**F**)

**Fig. 3** Effect of different antibiotics on the specific growth rate (%) 2 weeks after the of 24-h antibiotic treatment. Data are shown as mean  $\pm$  SE ( $n = 14$ ). Note that the \* indicates treatments with the lowest  $p$  values in the multiple comparison with the control group (ANOVA,  $p = 0.049$ )



**Table 2** Halogenated natural products observed in *Asparagopsis* tissue. The key compounds quantified are in bold and in order of elution on the chromatogram

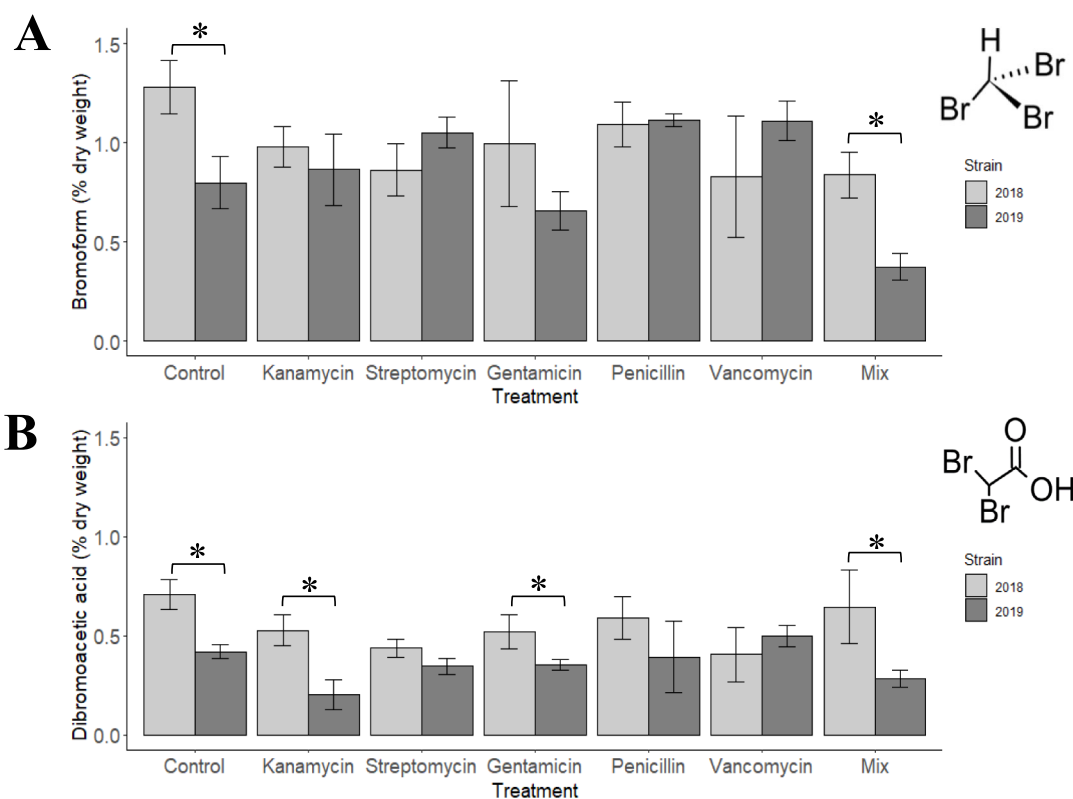
| Compound                                                   | Formula                                      | Retention time (min) |
|------------------------------------------------------------|----------------------------------------------|----------------------|
| Dibromochloromethane (DBCM)                                | $\text{CHBr}_2\text{Cl}$                     | 3.51                 |
| <b>Bromoform (Tribromomethane)</b>                         | <b><math>\text{CHBr}_3</math></b>            | <b>4.38</b>          |
| Bromochloroacetic acid (BCAA)                              | $\text{BrClCHCOOH}$                          | 4.62                 |
| <b>Dibromoacetic acid (DBAA)</b>                           | <b><math>\text{Br}_2\text{CHCOOH}</math></b> | <b>5.36</b>          |
| Carbon tetrabromide (CT)                                   | $\text{CBr}_4$                               | 6.02                 |
| 3,3,-Dibromoprop-2-enoic acid                              | $\text{C}_3\text{H}_2\text{Br}_2\text{O}_2$  | 6.40                 |
| Naphthalene                                                | $\text{C}_{10}\text{H}_8$                    | 7.02                 |
| 1,1,3-Tribromopropan-2-one (1,1,3-Tribromoacetone)         | $\text{C}_3\text{H}_3\text{Br}_3\text{O}$    | 7.25                 |
| 1,1,3,3-Tetrabromopropan-2-one (1,1,3,3-Tetrabromoacetone) | $\text{C}_3\text{H}_2\text{Br}_4\text{O}$    | 8.66                 |

bromoform was approximately 1% of the dry weight of the seaweed, almost double the concentration of DBAA (Fig. 4).

Across all treatments and strains, bromoform concentration ranged from 0.205 to 1.983% dw, while DBAA ranged

from 0.035 to 1.383% dw (Fig. 4). The statistical analyses of bromoform and DBAA concentrations across S-2018 and S-2019 revealed significant interactions for both compounds, with antibiotic- and strain-specific effects (Table 3, Treatment  $\times$  Strain interactions for both compounds,  $p = 0.028$ – $0.032$ ). The significant interaction terms were driven by different factors for the compounds: bromoform in general was affected by antibiotic treatment, with the lowest concentration in the mixed dose of antibiotics for both strains (Fig. 4A). However, for all single antibiotic treatments there tended to be lower bromoform compared to the control for S-2018, whereas there was no clear trend for S-2019, albeit with most single antibiotic treatments higher than the control (Fig. 4A). DBAA concentration was typically twice as high in S-2018 except for vancomycin treatment in which S-2019 had higher DBAA (Fig. 4B). The key treatment comparisons driving the significant interactions for bromoform, and DBAA are presented separately for each natural product in the following paragraphs.

Antibiotic treatments significantly influenced bromoform concentration, the main halogenated compound, with vancomycin and penicillin driving bromoform concentrations to or



**Fig. 4** Concentrations of halogenated compounds in *Asparagopsis taxiformis* (% dry weight). Barplot (A) illustrates the bromoform content, while (B) depicts the dibromoacetic acid (DBAA) content. Data are presented as mean  $\pm$  SE. Bars represent the two *Asparagopsis taxiformis* strains, dark bars denote S-2018, whereas lighter bars denote S-2019. Bars with \* indicate key differences (effect sizes of

20%–50%) between strains across treatments contributing to significant interaction terms (2-factor ANOVAs, Table 3). The compounds were extracted using solid–liquid extraction with methanol and naphthalene (30.1 ppm) as the internal standard. Extracts were analysed using a PerkinElmer Clarus 580 Gas Chromatograph (GC) coupled with a PerkinElmer Clarus SQ8S Mass Spectrometer (MS)

**Table 3** A 2-factor ANOVA output for the concentration of halogenated natural products. Key significant results in bold comparing the effects of antibiotic ‘treatment’ on two *Asparagopsis* cultures (‘strain’). ‘Df’ = degrees of freedom; ‘F’ = F-statistic;  $p = p\text{-value}$

|                    | Df | Bromoform |              | Dibromoacetic acid |              |
|--------------------|----|-----------|--------------|--------------------|--------------|
|                    |    | F         | <i>p</i>     | F                  | <i>p</i>     |
| Treatment          | 6  | 2.52      | 0.032        | 1.85               | 0.106        |
| Strain             | 1  | 2.22      | 0.142        | 17.39              | < 0.001      |
| Treatment × Strain | 6  | 2.59      | <b>0.028</b> | 2.52               | <b>0.032</b> |
| Residuals          | 54 |           |              |                    |              |

exceeding 1% dry weight. For instance, under vancomycin, bromoform levels reached  $1.108\% \text{ dw} \pm 0.100 \text{ SE}$  in S-2019 and  $0.827\% \text{ dw} \pm 0.308 \text{ SE}$  in S-2018. Similarly, penicillin resulted in comparable concentrations between strains, with S-2019 reaching  $1.112\% \text{ dw} \pm 0.031 \text{ SE}$  and S-2018  $1.091\% \text{ dw} \pm 0.115 \text{ SE}$ . Gentamicin also led to notable bromoform levels, approaching 1% dw in S-2018 ( $0.995\% \text{ dw} \pm 0.318 \text{ SE}$ ), though levels remained lower in strain S-2019 ( $0.656\% \text{ dw} \pm 0.095 \text{ SE}$ ). The mixed treatment for S-2018, the one with highest concentrations of bromoform, was close to streptomycin and vancomycin ( $\sim 0.84\% \text{ dw}$ ). Nonetheless, in S-2019 the bromoform concentrations were much lower, with an average of  $0.372\% \text{ dw} \pm 0.067 \text{ SE}$  far away from the others average ( $\sim 0.931\% \text{ dw}$ ).

The influence of antibiotic treatments on DBAA was more complex across both treatments and strains. S-2018 showed higher concentrations than S-2019, with an overall concentration  $\sim 35\%$  higher across all treatments, particularly under the control, gentamicin, kanamycin, and penicillin. Specifically, the control resulted in a  $\sim 41\%$  higher concentration in S-2018 compared to S-2019 ( $0.708\% \text{ dw} \pm 0.077 \text{ SE}$  and  $0.420\% \text{ dw} \pm 0.034 \text{ SE}$ , respectively), reflecting a significant difference in DBAA concentrations between the two strains. S-2018 exhibited more consistent results compared to S-2019, which showed greater variability, and lower concentrations of DBAA. For instance, S-2019 had lower concentrations especially under the kanamycin treatment ( $0.204\% \text{ dw} \pm 0.076 \text{ SE}$ ). However, vancomycin had a similar response in both strains, with S-2018, ( $0.500\% \text{ dw} \pm 0.060 \text{ SE}$ ) and S-2019 ( $0.405\% \text{ dw} \pm 0.139 \text{ SE}$ ). When comparing the concentrations following gentamicin and streptomycin treatments, S-2018 had a higher average concentration ( $0.520\% \text{ dw} \pm 0.086 \text{ SE}$  and  $0.437\% \text{ dw} \pm 0.046 \text{ SE}$ ) compared to S-2019 ( $0.355\% \text{ dw} \pm 0.027 \text{ SE}$  and  $0.346\% \text{ dw} \pm 0.041 \text{ SE}$ ). The mixed treatment was lower in S-2019 ( $0.283\% \text{ dw} \pm 0.039 \text{ SE}$ ), not differing much from kanamycin (lowest concentration for that strain), with not much effect for S-2018.

In summary, across treatments, S-2018 mostly exhibited higher concentrations of both bromoform (4 of 7 treatments: Fig. 4a) and DBAA (6 of 7 treatments: Fig. 4b) compared to S-2019. On average, across both strains and all treatments, DBAA levels were approximately 50% of bromoform concentrations. While bromoform concentrations were notably higher under vancomycin and penicillin, DBAA levels remained below 1% dw but displayed variability across treatments.

## Discussion

Although epiphytic bacteria can play essential roles in seaweed physiology including provision of some vitamins (Marshall et al. 2006), some may also exert pathogenic effects that are not yet well characterised. These relationships have significant commercial implications, particularly for the emerging seaweed farming industry focused on the genus *Asparagopsis*, which is valued for its potential to reduce methane emissions in cattle (Kinley et al. 2016; Li et al. 2018), enhance skincare formulations (Januário et al. 2024), and improve fish immune systems (Thépot et al. 2022). Despite its importance, little is known about whether the bacteria on the surface of *Asparagopsis* influence the growth and production of bioactive halogenated compounds. In this study, antibiotics with different modes of action were applied to disrupt the epiphytic bacteria on the filamentous stage of *Asparagopsis* under intensive culture at a stocking density of  $0.5 \text{ g fresh weight L}^{-1}$ . Using fluorescence microscopy, we demonstrated that gentamicin and streptomycin were most effective and reduced bacterial density from  $\sim 1 \text{ per } 32 \mu\text{m}^2$  to  $\sim 1 \text{ per } 260 \mu\text{m}^2$ . This reduction correlated with a modest positive effect on *Asparagopsis* growth but negatively impacted the bromoform and DBAA concentrations, highlighting potential trade-offs in culture management involving bacterial density, growth, and metabolite production in *Asparagopsis*. Moreover, the presence of vitamins in enriched culture media may mask auxotrophic dependencies making it difficult to fully assess bacterial contributions.

Specific bacterial taxa that persisted after an 86–89% reduction in bacterial density, might be critical for maintaining optimal growth and metabolite production. Such intricate relationships between microbial symbionts and their host have been observed in different seaweed systems. For instance, studies on *Ulva* spp. have shown that bacterial symbionts are vital for maintaining normal growth and morphology (Marshall et al. 2006). Disrupting these bacterial communities through environmental stressors or antibiotics often alters the microbial composition, impacting

algal physiology (Longford et al. 2019). Similarly, the antibiotic treatments in this study may have caused such shifts, explaining the observed growth enhancement if pathogenic bacteria had been reduced. Controlling pathogen infection is vital for the success of seaweed aquaculture, with beneficial bacteria protecting the seaweed from pathogens by producing antimicrobial compounds and supporting the microbiome in a way that prevents opportunistic or external pathogens proliferating or becoming virulent (Munang'andu 2016; Zhao et al. 2018; Li et al. 2022; Macrae and Vickers 2024). The fact that there was a significant reduction in bacterial density with no, or even positive, impact on growth of the seaweed also suggests that any symbiosis with specific bacteria does not require significant densities of beneficial bacteria, and that probably higher densities could affect the host. In a study by Zhang et al. (2022) they observed that biofilm on seagrass leaves in the species *Zostera marina* led to higher oxygen concentrations and pH, which in turn increases carbon dioxide limitation and diminishes photosynthetic efficiency. In our study, biofilm formation was observed (see Fig. 2A and C).

Based on the bacterial density results, a 24-h antibiotic bath with vancomycin, streptomycin and gentamicin maintained a low density of bacteria over a two-week period, with no detectable impact on *Asparagopsis* growth. The experiment duration (i.e. two weeks) does limit conclusions about whether the low bacterial densities in these treatments would be maintained and, if so, whether there may be long-term impacts of antibiotic treatment. However, we note that our domesticated cultures of *Asparagopsis* have significantly lower diversity than wild samples (Zhao et al. 2022) and that these cultures have been maintained for years. The bacterial densities we observed in untreated controls ( $\sim 0.031$  bacteria  $\mu\text{m}^{-2}$ ) were lower than those reported for wild species. For example, densities ranging from 0.3 to 1.5 bacteria  $\mu\text{m}^{-2}$  have been recorded on *Palmaria palmata*, *Laminaria digitata*, *Fucus serratus* and *Fucus spiralis* (Armstrong et al. 2000) and  $8.3 \times 10^{-6}$  to 0.1 bacteria  $\mu\text{m}^{-2}$  for wild *Laminaria hyperborea* (Bengtsson et al. 2010). Additionally, spatial variability on the sporophyte filament emerged as a significant factor, as bacterial densities differed across sampling locations, consistent with patterns observed in *Ulva australis*, where bacterial densities increase from thallus tips to the base (Egan et al. 2013). Furthermore, previous research demonstrated significantly higher epiphytic bacterial densities in *Asparagopsis armata* occur on algae that no longer produce halogenated metabolites, and that bromoform and dibromoacetic acid were more active against bacteria isolated from algae lacking those compounds than those producing them (Paul et al. 2006). We also observed significant sample variability in the morphologically simple filaments and no evidence for changes in cell size.

Our results highlight the differential impact that antibiotic treatments can have on two different domesticated strains of *Asparagopsis*. For example, the statistical analyses of bromoform and DBAA concentrations revealed significant interactions for both compounds, with antibiotic- and strain-specific effects. These findings suggest that genetic factors may influence halogenated metabolite production more than bacterial density (Paul and Puglisi 2004; Sudatti et al. 2021). Firstly, we did not find any evidence for the direct negative impact of the antibiotics on the concentrations of the halogenated compounds, even for the mixed antibiotic treatment with essentially 5-times the dose (no cell discolouration, minor 10% reduction in bromoform, but not DBAA). An alternative explanation for the reduction in halogenated compound concentrations across the antibiotic treatment is that the need for chemical defence, by releasing the compounds to the surface (Paul et al. 2006), was reduced with the significant reduction in bacterial density. Regardless, the differences between the two strains were  $\sim 3.5$  times more than the differences between antibiotics with the S-2018 consistently produced higher bromoform and DBAA concentrations. The results suggest that strain plays a critical role in the production of DBAA, whereas both treatment and strain interactions influence bromoform production. The significant compound interactions highlighted the complexity of the response, where the impact of treatments differs between strains. While *Asparagopsis* is known to make over 100 halogenated compounds (McConnell and Fenical 1977), many remain uncharacterised, necessitating further research into their ecological significance and function. Moreover, natural products from seaweeds demonstrate diverse antibacterial properties, with some compounds selectively targeting bacterial communities, such as furanones from *Delisea pulchra* and polyhalogenated 2-heptanones from *Bonnemaisonia hamifera*, while others, like fucoxanthin, inhibit bacterial attachment non-selectively (Saha et al. 2011).

Future research should focus on the long-term effects of microbiome manipulation, including fungi, archaea and other microorganisms of the microbiome, to optimise seaweed cultivation for growth, resilience, and bioactive compound production. This should consider both aspects of managing microbes in seaweed cultivation, reducing the prevalence of pathogenic (Ward et al. 2020; Spagnuolo and Genovese 2024) or enhancing the prevalence of symbiotic or competitively beneficial bacteria (Marshall et al. 2006; Tapia et al. 2016), and the more targeted understanding of whether there are specific symbioses between certain epiphytic bacteria and growth or metabolites. Harnessing the understanding of how to regulate the seaweed microbiome in aquaculture presents significant potential for enhancing pathogen resistance, ensuring the stability of a beneficial microbiome which is vital for the success of seaweed aquaculture.

## Conclusion

In conclusion, this study highlights the complex relationship between epiphytic bacteria and *Asparagopsis* tetrasporophyte growth and metabolite production. Antibiotic treatments successfully reduced bacterial densities, which led to a slight increase in growth, but simultaneously decreased the production of the key bioactive bromoform. These results suggest that the manipulation of bacterial communities and specific bacterial taxa are potentially playing a crucial role in the host. Future research should explore the long-term effects of bacterial community shifts, as well as the role of other compounds like carbon tetrabromide, bromochloroacetic acid, and haloacetones, in the sustainable cultivation of *Asparagopsis* and other seaweeds. Understanding these dynamics is essential for optimising growth and bioactive compound production in commercial seaweed farming systems. This includes the exploration of potential positive symbioses between bacteria and seaweeds that enhance production traits, noting that even a small population of “good” bacteria (i.e., < 1 bacterium per 260  $\mu\text{m}^2$ ) might be all that is required for intensive cultivation, presumably to meet the nutritional needs (e.g., vitamins and reduced nitrogen), which are crucial for seaweed metabolic processes (Croft et al. 2005; Zehr and Capone 2020).

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10811-025-03530-z>.

**Author's contributions** S.B.: Writing – original draft, Formal analysis, Data curation, Conceptualization. A.H.C.: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. S.F.C.: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. D.A.H.: Writing – review & editing. N.A.P.: Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

**Funding** Open Access funding enabled and organized by CAUL and its Member Institutions. This work has been supported by an Australian Research Council Discovery Project to S.F.C, N.A.P and A.H.C (DP200103013).

**Data availability** Data are available upon reasonable request.

## Declarations

**Competing interests** The authors declare no competing interests.

**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

- Abbott DW, Aasen IM, Beauchemin KA, Grondahl F, Gruninger R, Hayes M, Huws S, Kenny DA, Krizsan SJ, Kirwan SF, Lind V, Meyer U, Ramin M, Theodoridou K, Dv S, Walsh PJ, Waters S, Xing X (2020) Seaweed and seaweed bioactives for mitigation of enteric methane: challenges and opportunities. *Animals* 10:2432
- Armstrong E, Rogerson A, Leftley JW (2000) The abundance of heterotrophic protists associated with intertidal seaweeds. *Estuar Coast Shelf Sci* 50:415–424
- Bengtsson MM, Sjøtun K, Øvreås L (2010) Seasonal dynamics of bacterial biofilms on the kelp *Laminaria hyperborea*. *Aquat Microb Ecol* 60:71–83
- Brugman S, Ikeda-Ohtsubo W, Braber S, Folkerts G, Pieterse CMJ, Bakker PAHM (2018) A comparative review on microbiota manipulation: lessons from fish, plants, livestock, and human research. *Front Nutr* 5:80–80
- Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG (2005) Algae acquire vitamin B<sub>12</sub> through a symbiotic relationship with bacteria. *Nature* 438:90–93
- Egan S, Harder T, Burke C, Steinberg P, Kjelleberg S, Thomas T (2013) The seaweed holobiont: understanding seaweed–bacteria interactions. *FEMS Microbiol Rev* 37:462–476
- Gao Y, Chen Z, Yao W, Li D, Fu X (2021) Gentamicin combined with hypoionic shock rapidly eradicates aquaculture bacteria *in vitro* and *in vivo*. *Front Microbiol* 12:641846
- Ghaderiadekani F, Quartino ML, Wichard T (2020) Microbiome-dependent adaptation of seaweeds under environmental stresses: a perspective. *Front Mar Sci* 7:575228
- Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Can J Microbiol* 8:229–239
- Hruby K, Schiel H (1996) Antimicrobials. In: Descotes J (ed) *Human Toxicology*. Elsevier Science, Amsterdam, pp 429–438
- Januário AP, Félix C, Félix R, Shiels K, Murray P, Valentão P, Lemos MFL (2024) Exploring the therapeutic potential of *Asparagopsis armata* biomass: a novel approach for acne vulgaris treatment. *Mar Drugs* 22:489
- Kinley RD, De Nys R, Vucko MJ, MacHado L, Tomkins NW (2016) The red macroalgae *Asparagopsis taxiformis* is a potent natural antimethanogenic that reduces methane production during *in vitro* fermentation with rumen fluid. *Anim Prod Sci* 56:282–289
- Klein Jan H, Jeanthon C, Boyen C, Dittami SM (2017) Exploring the cultivable *Ectocarpus* microbiome. *Front Microbiol* 8:2456
- Lachnit T, Blümel M, Imhoff JF, Wahl M (2009) Specific epibacterial communities on macroalgae: phylogeny matters more than habitat. *Aquat Biol* 5:181–186
- Lewin J (1966) Silicon metabolism in diatoms. V. Germanium dioxide, a specific inhibitor of diatom growth. *Phycologia* 6:1–12
- Li X, Norman HC, Kinley RD, Laurence M, Wilmot M, Bender H, De Nys R, Tomkins N (2018) *Asparagopsis taxiformis* decreases enteric methane production from sheep. *Anim Prod Sci* 58:681–688
- Li J, Majzoub ME, Marzinelli EM, Dai Z, Thomas T, Egan S (2022) Bacterial controlled mitigation of dysbiosis in a seaweed disease. *ISME J* 16:378–387
- Li J, Weinberger F, de Nys R, Thomas T, Egan S (2023) A pathway to improve seaweed aquaculture through microbiota manipulation. *Trends Biotechnol* 41:545–556
- Longford SR, Campbell AH, Nielsen S, Case RJ, Kjelleberg S, Steinberg PD (2019) Interactions within the microbiome alter microbial interactions with host chemical defences and affect disease in a marine holobiont. *Sci Rep* 9:1363–1363
- Machado L, Magnusson M, Paul NA, de Nys R, Tomkins N (2014) Effects of marine and freshwater macroalgae on *in vitro* total gas and methane production. *PLoS ONE* 9:e85289

- Machado L, Magnusson M, Paul NA, Kinley R, de Nys R, Tomkins N (2016) Identification of bioactives from the red seaweed *Asparagopsis taxiformis* that promote antimethanogenic activity in vitro. *J Appl Phycol* 28:3117–3126
- Macrae OC, Vickers CJ (2024) The significance of microbiota in New Zealand seaweed aquaculture. *N Z J Mar Freshw Res* 59:509–536
- 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*. *Microb Ecol* 52:302–310
- McConnell O, Fenical W (1977) Halogen chemistry of the red alga *Asparagopsis*. *Phytochemistry* 16:367–374
- McLawn RW (2012) Guidelines for the monitoring of vancomycin, aminoglycosides and certain antibiotics. In: Dasgupta A (ed) *Therapeutic drug monitoring*. Elsevier, Boston, pp 197–218
- Montalban-Arques A, De Schryver P, Bossier P, Gorkiewicz G, Mulero V, Gatlin DM, Galindo-Villegas J (2015) Selective manipulation of the gut microbiota improves immune status in vertebrates. *Front Immunol* 6:512
- Munang'andu HM (2016) Environmental viral metagenomics analyses in aquaculture: applications in epidemiology and disease control. *Front Microbiol* 7:1986
- Papich M (2015) *Saunders handbook of veterinary drugs*. Elsevier, St Louis, pp 425–426
- Paul VJ, Puglisi MP (2004) Chemical mediation of interactions among marine organisms. *Nat Prod Rep* 35:189–209
- Paul NA, De Nys R, Steinberg PD (2006) Chemical defence against bacteria in the red alga *Asparagopsis armata*: linking structure with function. *Mar Ecol Prog Ser* 306:87–101
- Peixoto RS, Rosado PM, Leite DCdA, Rosado AS, Bourne DG (2017) Beneficial microorganisms for corals (BMC): proposed mechanisms for coral health and resilience. *Front Microbiol* 8:341
- Reeves D, Lovering A, Thomson A (2016) Therapeutic drug monitoring in the past 40 years of the *Journal of Antimicrobial Chemotherapy*. *J Antimicrob Chemother* 71:3330–3332
- Roque BM, Venegas M, Kinley RD, De Nys R, Duarte TL, Yang X, Kebreab E, Wells JE (2021) Red seaweed (*Asparagopsis taxiformis*) supplementation reduces enteric methane by over 80 percent in beef steers. *PLoS One* 16:e0247820
- Rosado PM, Leite DCA, Duarte GAS, Chaloub RM, Jospin G, Nunes da Rocha U, Saraiva PJ, Dini-Andreote F, Eisen JA, Bourne DG, Peixoto RS (2019) Marine probiotics: increasing coral resistance to bleaching through microbiome manipulation. *ISME J* 13:921–936
- Saha M, Rempt M, Grosser K, Pohnert G, Weinberger F (2011) Surface-associated fucoxanthin mediates settlement of bacterial epiphytes on the rockweed *Fucus vesiculosus*. *Biofouling* 27:423–433
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671
- Singh RP, Reddy CRK (2014) Seaweed–microbial interactions: key functions of seaweed-associated bacteria. *FEMS Microbiol Ecol* 88:213–230
- Spagnuolo D, Genovese G (2024) Macroalgal diseases: exploring biology, pathogenesis, and management strategies. *Phycology* 4:450–464
- Sudatti DB, Oliveira AS, da Gama BAP, Fujii MT, Rodrigues SV, Pereira RC (2021) Variability in seaweed chemical defense and growth under common garden conditions. *Front Mar Sci* 8:3389
- Tapia JE, González B, Goulitquer S, Potin P, Correa JA (2016) Microbiota influences morphology and reproduction of the brown alga *Ectocarpus* sp. *Front Microbiol* 7:197
- Team RC (2021) R: A language and environment for statistical computing. <https://www.R-project.org/>
- Thépot V, Campbell AH, Rimmer MA, Jelocnik M, Johnston C, Evans B, Paul NA (2022) Dietary inclusion of the red seaweed *Asparagopsis taxiformis* boosts production, stimulates immune response and modulates gut microbiota in Atlantic salmon *Salmo salar*. *Aquaculture* 546:737286
- Vardanyan RS, Hrubby VJ (2006) *Synthesis of essential drugs*. Elsevier, Amsterdam
- Ward GM, Faisan JP, Cottier-Cook EJ, Gachon C, Hurtado AQ, Lim PE, Matoju I, Msuya FE, Bass D, Brodie J (2020) A review of reported seaweed diseases and pests in aquaculture in Asia. *J World Aquac Soc* 51:815–828
- Woolard FX, Moore RE, Roller PP (1979) Halogenated acetic and acrylic acids from the red alga *Asparagopsis taxiformis*. *Phytochemistry* 18:617–620
- Xavier R, Severino R, Silva SM (2024) Signatures of dysbiosis in fish microbiomes in the context of aquaculture. *Rev Aquac* 16:706–731
- Zehr JP, Capone DG (2020) Changing perspectives in marine nitrogen fixation. *Science* 368:eaay9514
- Zhang Q, Kühl M, Brodersen KE (2022) Effects of epiphytic biofilm activity on the photosynthetic activity, pH and inorganic carbon microenvironment of seagrass leaves (*Zostera marina* L.). *Front Mar Sci* 9:835381
- Zhao Z, Pan Y, Jiang J, Gao S, Sun H, Dong Y, Sun P, Guan X, Zhou Z (2018) Unrevealing variation of microbial communities and correlation with environmental variables in a full culture-cycle of *Undaria pinnatifida*. *Mar Environ Res* 139:46–56
- Zhao M, Campbell A, Patwary Z, Wang T, Lang T, Webb J et al (2022) The red seaweed *Asparagopsis taxiformis* genome and integrative -omics analysis. *R Square*. <https://doi.org/10.21203/rs.3.rs-2232367/v1>
- Zozaya-Valdés E, Roth-Schulze AJ, Thomas T (2016) Effects of temperature stress and aquarium conditions on the red macroalga *Delisea pulchra* and its associated microbial community. *Front Microbiol* 7:161

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