The Plymouth Student Scientist - Volume 16 - 2023 The Plymouth Student Scientist - Volume 16, No.2 - 2023

2023

Diversity and bioactive potential of leaf-, and root sediment-associated bacteria from Zostera marina in the Yealm Estuary, Southwest England

Capps, E.

Capps, E. (2023) 'Diversity and bioactive potential of leaf-, and root sediment-associated bacteria from Zostera marina in the Yealm Estuary, Southwest England', The Plymouth Student Scientist, 16(2), pp. 132-156.
https://pearl.plymouth.ac.uk/handle/10026.1/21843

The Plymouth Student Scientist
University of Plymouth

*The Plymouth Student Scientist, 2023, **16**, (2), 132-156*

Diversity and bioactive potential of leaf-, and root sediment-associated bacteria from *Zostera marina* in the Yealm Estuary, Southwest England

Eva Capps

Project adviser: Professor Mathew Upton School of Biomedical Sciences, University of Plymouth, Drake Circus, Plymouth, PL4 8AA.

Abstract

Increasing antimicrobial resistance (AMR) among human pathogenic bacteria demands urgent efforts to uncover novel natural products for developing new antibiotic drugs. Since terrestrial sources of natural products are overmined, attention in biodiscovery is shifting towards marine microbial ecosystems which are subject to unique environmental stressors. Resulting metabolic adaptations provide potential for potent antibacterial compounds. Thus, the microbiomes of seagrass components in *Zostera marina*, and the activity of culturable isolates against human pathogens, were explored in this study. The sample site used was in the Yealm Estuary, Southwest England, a location previously unexplored in this context. Seagrass leaves at 20cm and 50cm depths, and root-associated sediment, were collected and plated on selective and non-selective media. DNA was extracted from shallow depth leaves and sediment and purified for 16S amplicon surveying. A total of 207 isolates were subcultured and antibacterial activity of pure colonies was tested. In simultaneous antagonism tests, 26 isolates inhibited *Staphylococcus aureus*, and 41 inhibited *Escherichia coli*, with sediment-derived isolates having the most overall activity. In addition, the sediment microbiome had higher alpha diversity indices, and highest operational taxonomic units (OTUs) of genera *Bacillus* and *Sporosarcina,* which is consistent with findings in other studies. Predominant genera in the seagrass leaf from 20cm depth were *Marinomonas, Colwellia*, and *Winogradskyella.* This study revealed *Zostera marina* plants in Southwest England to be a rich source of bacteria, including actinobacteria, with potent secondary metabolites which demonstrates the value in exploring new environments for bioactive molecules with pharmaceutical potential.

Keywords: Antimicrobial resistance (AMR), antibiotic discovery, natural products, marine bacteria, Zostera marina, seagrass microbiome, microbial diversity, microbial composition, 16S sequencing, actinobacteria, bioactivity, colony iridescence, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Staphylococcus aureus, Micrococcus luteus, Enterococcus faecalis.*

Introduction

The accumulation of bacterial antimicrobial resistance (AMR) poses an increasing threat to global public health and is predicted by one report to cause 10 million deaths per year by 2050 (O'Neill, 2016). Recent systematic analyses of data on AMR-associated burden predicts than in 2019 alone, there were 4.95 million AMR-associated deaths, 1.27 million of which were directly attributable to bacterial AMR (Murray et al., 2022). Since their discovery, antibiotics have been a critical component of modern medicine. However, waning efficacy due to the rise of AMR, including bacteria such as MRSA (methicillin resistant *Staphylococcus aureus*) necessitates the detection of novel antimicrobial compounds, and the development of multifaceted treatment and stewardship strategies to optimise and retain their value. Resistance to antibiotics was documented by Alexander Fleming in 1948 shortly after he discovered penicillin, so this is not an emerging issue, but a neglected one. The subsequent misuse and overuse of these drugs in clinical and agricultural settings, and ignorance to Fleming's warning has contributed to the current levels of resistance and health crises (Dadgostar, 2019).

Natural products are complex chemical compounds produced by living organisms to enhance their survival. The exploration and manipulation of their properties has allowed the creation of many pharmaceutical drugs, antimicrobial and otherwise (Dzobo, 2022). Traditional methods of natural product discovery relied on mining bacteria from soil, and using phenotypic techniques to systematically screen them for activity. From 1940 to the 1960s, many natural products and their semisynthetic derivatives made it to market this way, and scaffolded pathways to further discovery, e.g., fluoroquinolones (Fischbach and Walsh, 2009). Movement towards the target-based high throughput screening of synthetic products offered a promising alternative to these methods, which had high rates of metabolite rediscovery, but yielded poor results with no new structural classes of antibiotics discovered in nearly 40 years (Mills, 2006). As a consequence, reliable natural product discovery methods are popular again, this time using more diverse environmental samples and culture techniques to discover previously 'untapped' bacterial species and their metabolites (Boyd, Teng and Frei, 2021).

Marine environments are unique and diverse ecosystems which support a rich and dynamic variety of flora and fauna (Gagné et al., 2020). Marine bacteria have evolved adaptations to survive in chemical and physical conditions which are not found in these combinations elsewhere, like high pressure and salinity. Such environmental stressors have caused bacteria to develop specialised metabolic pathways, enabling the production of unique secondary metabolites (de Carvalho and Fernandes, 2010). The co-evolution of bacteria with other microorganisms furthers the survival pressure due to competition for resources, both within and between species, so the production of compounds which kill or inhibit competitors gives a major advantage (Bhatnagar and Kim, 2010). Many of these secondary metabolites have been found to inhibit some human pathogens, and are derived from a range of sources including seaweed, sea sponges, seagrasses, and coral reefs (Srinivasan et al., 2021). Actinobacteria are a phylum of bacteria found in marine samples, whose natural products have proven bioactive potential including antibacterial, antifungal, antiviral, and anti-inflammatory effects. Streptomycetacae are a family of actinobacteria whose secondary metabolites famously have activity against pathogens like *Staphylococcus aureus* (Girão et al., 2019). Being comprised

of such diverse microbial communities, and situated in an atypical environment for traditional screening, seagrasses are an example of a good candidate source for novel metabolites with antibacterial activity (Shang et al., 2018).

Seagrasses are flowering marine angiosperms and keystone species in underwater ecosystems of all continents except Antarctica. They are descendants of terrestrial plants which colonised the sea as long as 100 million years ago and have developed adaptations to life in the marine environment as a result (Olsen et al., 2016). The ecological benefits afforded by seagrass meadows are well documented as they support a wealth of biodiversity including invertebrates which feed on epiphytic bacteria in seagrass-associated sediment (Kharlamenko et al., 2001), and provide nurseries for many fish species in their leaves, at a critical stage in their lives (Lilley and Unsworth, 2014). Seagrass plants, their sediment, and their epiphytic bacteria also have complex involvements in biogeochemical cycles, chiefly through carbon sequestration, nitrogen fixation, and sulphur cycling (Sun et al., 2015). In addition, seagrass meadows are of high (and increasing) economic significance versus other marine biomes such as coral reefs (Costanza et al., 2014), due partly to their ability to modulate wave heights and current velocities (Reidenbach and Thomas, 2018), but also their role in the protection of fish which provide income for fisheries and employment in coastal areas (Lilley and Unsworth, 2014). Despite the recognised importance of their roles as ecosystem engineers, seagrasses are declining in coverage due to anthropogenic stressors such as coastal development, extreme climate events, and poor water quality (Green, Chadwick and Jones, 2018).

Seagrass plants and their associated sediments are home to rich microbial communities whose compositions influence the plant's growth and productivity, and are themselves shaped by habitat-filtering, nutrient availability, and plant metabolism (Fahimipour et al., 2017). The plant's microbiome as a whole is made up of three main habitats, with distinct microbial compositions: the phyllosphere (area above ground), endosphere (internal plant tissue), and rhizosphere (periphery of root tissue). These ecosystems, their roles, and interactions are described comprehensively in a recent review (Ugarelli et al., 2017). Microbial compositions of local sea water are important determinants of seagrass microbiomes and as a result, metabolic models show that these microbial communities undergo differing amounts of spatial turnover, offering different profiles of metabolites produced (Fahimipour et al., 2017). Despite the potential of seagrasses and their endophytes to produce antibacterial, antifungal, and cytotoxic metabolites being proven (Casella et al., 2013), there is still a lack of research on microbiomes and their relations to bioactivity, as the majority of previous studies have focussed on their symbiotic relationships and involvement in marine biogeochemistry. Alongside this, there are geographical disparities in knowledge and characterisation. For example, microbiomes of *Zostera marina* plants in the UK are altogether under characterised, especially in the context of their bioactive potentials, compared with species in more temperate waters (Fahimipour et al., 2017, Ghebretinsae et al., 2019). A better understanding of seagrass microbiomes and factors influencing them will not only allow a deeper exploration of their therapeutic potential, but aid conservation efforts which will allow this research to continue (Wang et al., 2020). To address current knowledge gaps, this project explores the leaf-, and sediment-associated microbiomes of *Zostera marina* in the Yealm Estuary, Southwest England, and the activity of isolated bacteria against common human pathogens.

Materials and Methods

Sample collection and processing

Samples of *Zostera marina* leaves at 20cm and 50cm depths, and root-associated sediment were collected from Cellar's Beach in the Yealm Estuary, Southwest England, (50°18'36"N 4°03'58"W) at a 0.5m (spring) low tide on 10th October 2022. See Figure 1 for location.

Salinity and water temperature (as factors known to influence microbial composition, abundance, and functioning) were measured at the collection site on the same tide on 9th November 2022. A YSI ProDSS Multiparameter water quality meter was used. Five water samples were collected for the measurement of copper concentration using inductively coupled plasma mass spectrometry (ICP-MS).

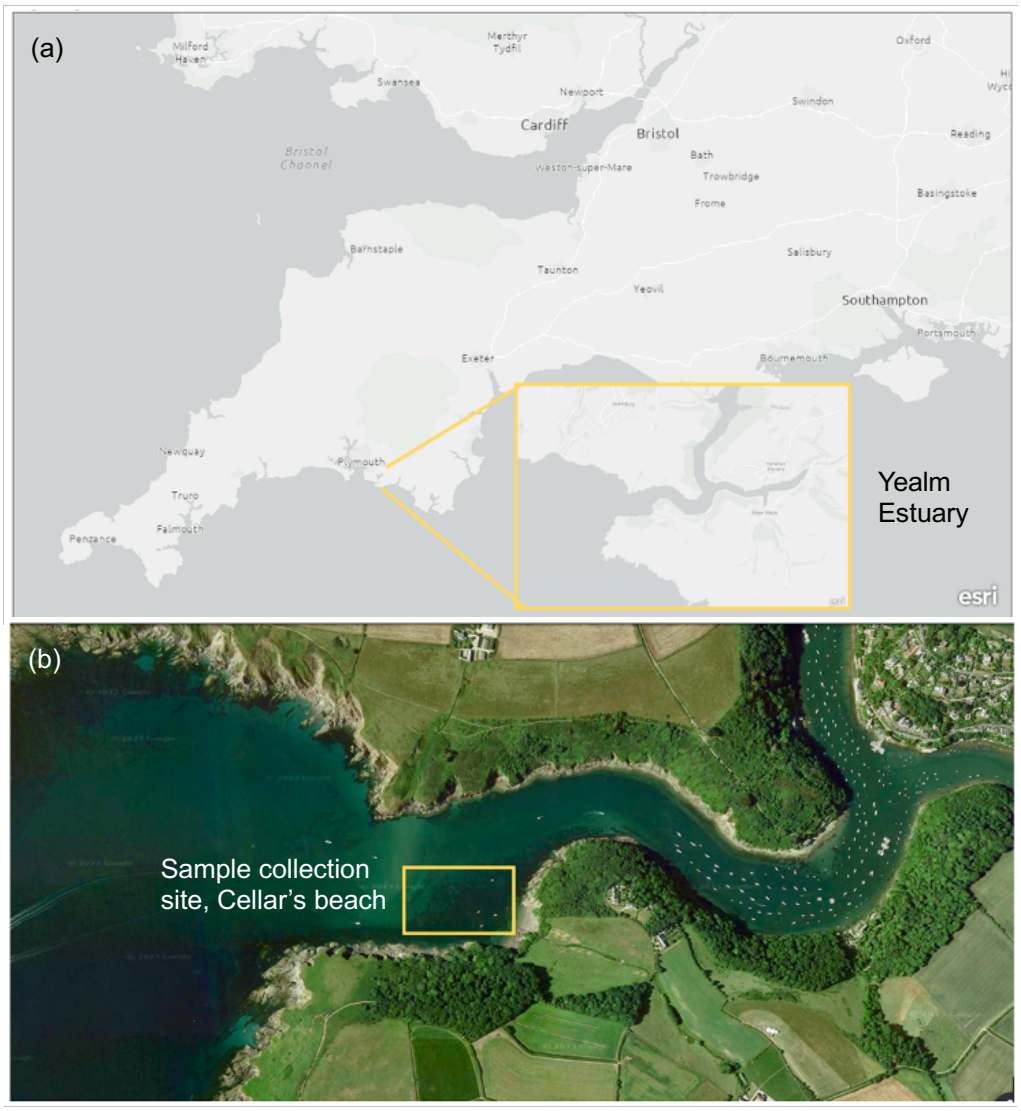


Figure 1: Location of the sample collection site, (a) relative to the Southwest of England, and (b), relative to the Yealm Estuary. Figure 1a adapted from the World Topographic Layer within ArcGIS Pro. Figure 1b adapted from Google Earth (2023).

Samples were labelled SGA (seagrass A, from fresh leaves at 20cm depth), SGB (seagrass B, from fresh leaves at 50cm depth), and RSD (root sediment, collected as close to the root as possible with plant *in situ* to not disturb it). To maintain the samples' compositions of epi- and endo-phytic bacteria at the time of collection, they were placed in sealed plastic bags and stored at 4°C until homogenisation and pre-treatment.

Approximately 1g (wet weight) of each sample was retrieved aseptically using sterile scissors and tweezers for leaves, and a spatula for sediment. Leaf samples were macerated with a pestle and mortar, and all samples underwent a 10mL ten-fold serial dilution with artificial seawater (ASW) Instant Ocean© from 10^{-1} to 10^{-5}. Autoclaved growth media (121°C for 15 minutes) used were R2A (Reasoner's 2A agar, OXOID), actinomycete (ACT) agar (Sigma-Aldrich), and marine agar (MA) (Millipore Marine Broth (MB) and agar powder (Thermo Scientific)). In addition to growth selection by agar nutrient composition, fungal growth inhibition was achieved using cycloheximide (30µg/mL) and nystatin (25µg/mL) in all media. Selectivity was further increased by adding 10µg/mL nalidixic acid to ACT and one half of MA, labelled MA(-NA), giving four final growth media; R2A, MA(-NA), ACT, MA(+NA). Sample dilutions for media with higher selectivity (ACT and MA(+NA)) were heat-treated (55°C water bath for 30min) prior to plate inoculation. Following spread plating of each dilution for each sample, media with greater selectivity were incubated at 28°C and media with lower selectivity (R2A, MA(-NA)) were incubated at room temperature, around 20°C.

Bacterial isolation

Bacterial growth was first assessed after six days. A lack of consistency in growth across dilutions for RSD prompted repeat dilutions and plating, labelled RSD(2). For all spread plates, colonies of diverse morphology were given a unique ID and described in terms of size, colour, texture, elevation, form, and margin (Supplementary Table 1). Colonies were re-streaked on their original media to obtain a pure culture.

Screening for activity

Isolates were grouped into testing batches based on media type and purity (impure colonies re-streaked), and screened for activity against six pathogens; three Gram negatives: *Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*, and three Gram positives: *Staphylococcus aureus, Micrococcus luteus,* and *Enterococcus faecalis.* Three high-throughput, low-cost assays were used.

Simultaneous antagonism

To test competitive exclusion, potential producers and pathogens were inoculated simultaneously onto non-specific growth media according to the protocol outlined by Tagg and Bannister (1979). Test pathogens were inoculated in 5mL Mueller Hinton Broth, MHB (Millipore) and grown at 37°C, 160rpm overnight. Using a 1 in 10 dilution of the pathogens with MHB (to increase accuracy), absorbance at 600nm was measured with a spectrophotometer then multiplied by ten for the original culture absorbance. Absorbance was used to calculate the pathogen volume needed in a 1mL solution with MHB, based on a required optical density (OD_{600}) value of 0.05 for all pathogens except *M.luteus* which used 0.10 (Beal et al., 2020). OD-adjusted pathogens were spread on square plates of MHA (Mueller Hinton Agar, Millipore)

divided into squares of 20x20mm. Potential producers were inoculated from solid media onto the middle of each square using autoclaved cocktail sticks. Plates were incubated at 37°C for 24 hours then results recorded.

Cross-streak
The cross-streak method of activity testing was carried out as described by Fernando Carvajal (1947) with the following adjustments: Potential producers were inoculated in a streak across the diameter of an MHA round plate and incubated at room temperature for seven days (as opposed to the three to five days) until there was a confluent line of growth and the isolate's stationary phase was reached. A suspension of each pathogen was created using 200µL ddH_2O (instead of streaking directly from cultures on solid media) then streaked perpendicular to the confluent isolate growth, without touching it, using a sterile 1µL loop. Plates were incubated at 37°C for 24 hours then checked for pathogen growth inhibition against a control plate.

Well-diffusion
Testing was based on the procedure outlined by Valgas et al., (2007). Single colonies of 24 active isolates were inoculated in 10mL of their corresponding broth (isolates from ACT and R2A in R2B (OXOID) and MA in MB) in a 50mL Erlenmeyer flask and incubated at 28°C and 160rpm for seven days until in their stationary phase. Pathogens for testing were inoculated in 5mL MHB and incubated **overnight at 37°C** and 160rpm. Dilutions were created to reach ODs as in the simultaneous antagonism. Solutions were spread on square MHA plates with 20x20mm grids and in the centre of each square, a borehole of 6mm diameter was created. From each liquid isolate culture, 1mL was centrifuged in an Eppendorf at 13,300rpm until a pellet formed. 50µL of isolate supernatant was added to the relevant well, with 50µL MHB in a control well. Plates were incubated at 37°C for 24 hours.

Isolate characterisation and nucleic acid analysis
A total of 24 isolates with activity were Gram stained according to Smith and Hussey, (2005), and photographed through a light microscope using oil immersion at 1000x. One isolate with strong activity on the simultaneous antagonism (ECSGA124) and one isolate with promising cross-streak activity (ECRSD(2)202) had their DNA purified (Zymo research protocol) and were further characterised by 16S rDNA PCR (using primers 27F and 1429R (Frank et al., 2008)) and Sanger sequencing. A consensus sequence was created by removing primers and aligning forward and reverse sequences. Database searching (BLAST and EZBioCloud) was carried out for species identification.

Microbiome characterisation
250mg of original samples SGA, SGB and RSD were processed using the Qiagen DNeasy® PowerSoil® Pro Kit according to the manufacturer's protocol (QIAGEN, 2021) then run through an IMPLEN NanoPhotometer® N60 to assess DNA purity. Two of these samples, SGA and RSD, were sent to Novogene for 16S Amplicon Surveying to ascertain bacterial community profiles (top ten) at phylum, class, order, family, and genus level.

Isolate storage
The existing 24 liquid cultures were used to create isolate stocks. For additional isolates of active, colourful, or iridescent natures, new liquid cultures were made by inoculating a single colony in 5mL MHB and incubating at 28°C and 160rpm for 48 hours. 750µL of each isolate's liquid culture was added to 750µL of 50% glycerol in a cryovial. Isolate stocks were created in duplicates and stored at -80°C.

Data presentation/analysis
Isolate activity spreadsheets were put into Minitab for data presentation. No assumptions/requirements for statistical comparisons of data sets were met.

Results
Environmental conditions
The mean temperature and salinity were calculated from five readings and were 13.18°C, and 20.73ppt (parts per thousand) respectively. Mean dissolved copper from five samples was 1.17µg/L.

Bacterial identification and phenotypic observations
A total of 207 colonies were streaked for isolation, 197 from original spread plates at the five different dilutions, and ten came from streaks of other isolates as co-isolates or contaminants. Of the 207, 79 came from SGA, 30 came from SGB, and 39 and 59 came from RSD and RSD(2) respectively (mean of 49 for both root sediment repeats). Phenotypic observations were recorded for each isolate, and included morphology, margin, shape, size, and colour (Supplementary Table 1, ST1). A diverse range of colours were observed, including orange, yellow, and pink. Some isolates from R2A (e.g., ECSGA44, ECSGA45, ECSGA98, ECSGA104) appeared pale and uniform in colour on the bench but showed iridescence when held up to the light on their original media (Supplementary Figure 1, SF1), and to a lesser extent when streaked on MHA, ACT, and MA. ECSGB105 had a similar iridescence but a more granular, metallic, appearance. ECSGB60 was pink in colour but had whiter areas on the edge of its streak, which were iridescent when held up to the light. Serial dilution plates from a pure colony of this isolate were pale pink in colour with angle-dependent iridescence, though angles were not measured (SF2). This iridescence is the result of the periodic arrangement of micron- and submicron-sized structures that interact with incident light to create a scattering of spectral colours which differ according to the angle of observation (Kientz et al., 2012).

Following initial tests, some isolates were carried forward for further testing based on their activity. These 24 isolates were Gram stained (Table 1).

Table 1: Gram stain results of isolates with promising activity which were carried forward for further investigation.

Sample ID †	Gram stain	Sample ID †	Gram stain	Sample ID †	Gram stain
ECSGA3	+	ECRSD63	+	ECSGA126	NA
ECSGA14	+	ECRSD64	+	ECSGA185	+
ECSGB20	+	ECRSD(2)91	+	ECSGB188	-
ECSGB25	+	ECSGA101	+	ECRSD195	+
ECSGA48	-	ECRSD(2)114	+	ECSGA200	-
ECSGA53	+	ECRSD(2)115	+	ECRSD(2)201	+
ECSGB57	NA	ECRSD(2)116	+	ECRSD(2)202	+
ECSGB60	+	ECSGA124	+	ECRSD(2)204	+

† Isolate names and table colours are coded by sample origin: Seagrass A (SGA) in blue, Seagrass B (SGB) in orange, Root sediment first dilution (RSD) in yellow, and Root sediment second dilution (RSD(2)) in green. A '+' Indicates a Gram positive isolate, '-' is a Gram-negative isolate, and 'NA' describes isolates that would require re-staining to get a result.

Two isolates with particularly promising activity, one in simultaneous antagonism (ECSGA124) and one in cross streaks (ECRSD(2)202), had their 16S rDNA PCR products sent for Sanger sequencing. Photos of Gram stains and colonies on their original media are included in SF3. The read lengths of this data are visible in Table 2, and BLASTn database search results from consensus sequences are in Table 3. Following a BLASTn search, EZBioCloud was used to confirm results. ECSGA124 was identified as *Micromonospora peucetia*, and ECRSD(2)202 was unidentifiable beyond genus level, *Streptomyces sp..*

Table 2: Data on forward, reverse, and consensus sequence length for both isolates that underwent 16S rDNA PCR and Sanger sequencing. Primers and areas of poorly defined peaks were removed to obtain the read lengths below.

	ECSGA124	ECRSD(2)202
Forward read length	998	1058
Reverse read length	1058	1078
Length of consensus sequence	963	775

Table 3: BLASTn database top hits, % Identity, and % Coverage for isolates nucleotide consensus sequences of ECSGA124 and ECRSD(2)202.

ECSGA124			ECRSD(2)202		
BLASTn top hits	% Identity	% Coverage	BLASTn top hits	% Identity	% Coverage
Micromonospora peucetia isolate Y70	99.79	100	*Streptomyces sp.* Strain AKT42	99.87	100
Micromonospora sp. Strain DT2-35	99.79	100	*Streptomyces sp.* Strain AKT12	99.87	100
Micromonospora peucetia strain DSM 43363	99.79	100	*Streptomyces sp.* Strain AKT11	99.87	100

Community analysis

DNA purity analysis yielded 260/280 absorbance ratios of 1.863 for SGA and1.569 for RSD. Taxonomic annotation according to Operational Taxonomic Units (OTUs) showed that bacteria from SGA were distributed across three main phyla: Proteobacteria, Bacteroidota, and Cyanobacteria (Figure 2a), and three main families: Marinomonodaceae, Colwelliaceae, and Flavobacteriaceae (Figure 2b). Predominant genera in SGA were *Marinomonas, Colwellia*, and *Winogradskyella* (Figure 2c). Bacteria from RSD were distributed across three main phyla: Proteobacteria, Firmicutes, and Actinobacteria, (Figure 2a) and two main families: Bacillaceae and Planococcaceae (Figure 2b). Predominant genera in RSD were *Bacillus* and *Sporosarcina* (Figure 2c). Many were still unidentified.

Alpha diversity indices Shannon and Simpson describe a higher microbial diversity in RSD than SGA, with marginally greater species evenness in SGA (using 1-D (Simpson's index)). With an increasing 1-D value, diversity increases, and the dominance of a few species is decreased. Chao1 and ACE, measures both give a higher degree of species richness to RSD. There was high sequencing coverage in both samples (Table 4). Beta diversity indices were calculated but results are not included as they factored in microbial communities in environmental samples from different working groups (e.g., sea sponges).

Table 4: Alpha diversity indices for operational taxonomic units (OTUs) with a clustering threshold of 97%. Shannon and Simpson are measures accounting for species richness and evenness, whereas Chao1 and ACE describe richness only. ACE = abundance-based coverage estimator.

	Seagrass A (SGA)	Root sediment (RSD)
Observed species	685	1179
Shannon	5.404	6.999
Simpson	0.932	0.954
Chao1	1170.484	1909.680
ACE	1144.495	1557.436
Goods Coverage	0.988	0.985

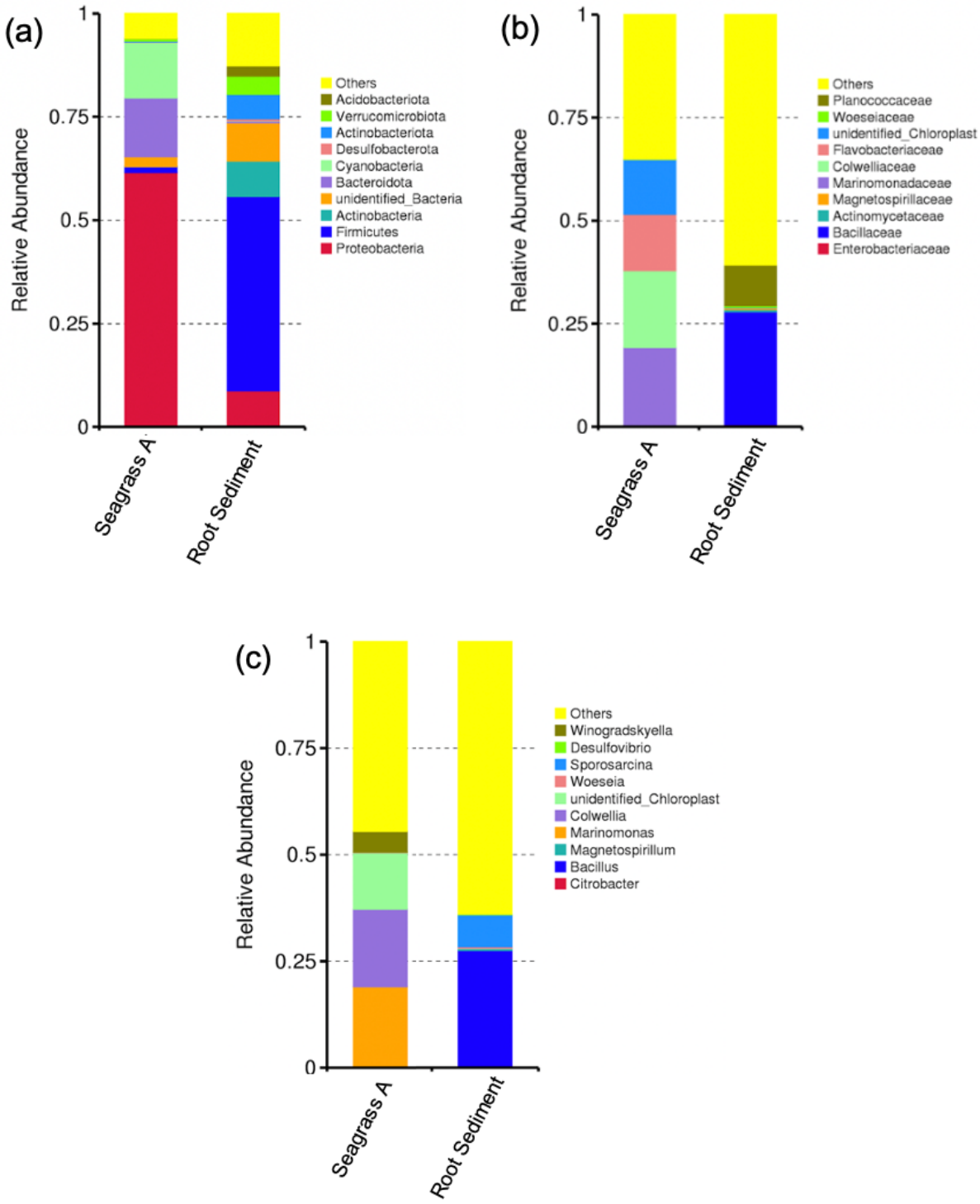


Figure 2: Relative abundances of top ten taxa at (a) phylum, (b) family, and (c) genus level. Figures generated by Novogene from 16S Amplicon QIIME Analysis results.

Bioactivity testing
Isolates of pure culture only were included for testing. Isolate plates which were contaminated, especially those that were swarmed, were excluded, and often from original spread plates of lower dilution.

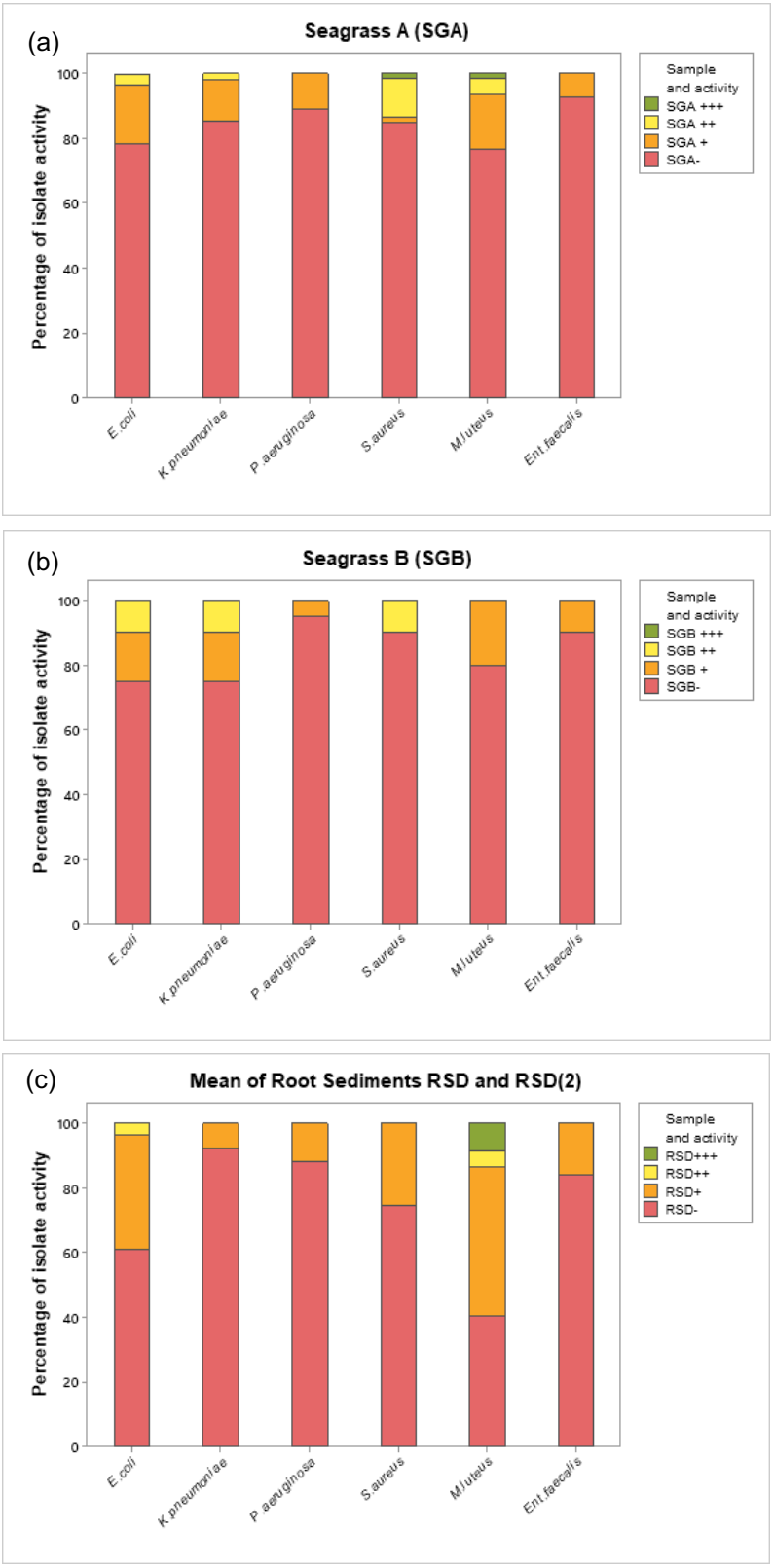
Simultaneous antagonism
A total of 139 isolates were included for testing. Of these, 46 originally came from MA(-NA), 50 came from R2A, 22 came from MA(-NA), and 21 came from ACT. 60 isolates were from SGA, 20 were from SGB, 21 were from the first RSD repeat, and 38 came from the second RSD repeat. Criteria for activity levels and examples can be found in SF4. Results showing activity at any level (including growth only) are available in ST2. Using original SGA and SGB data, and mean data for RSD and RSD(2), bar charts were produced to assess activity levels of each sample against the six pathogens tested. Combined mean RSD isolates show the highest proportions of isolates with activity of any level versus SGA and SGB (Figure 3), with the exception of *K.pneumoniae*, where activity is lower than for the seagrass-derived isolates.

Numbers of isolates with activity (including growth) per pathogen are: 41 for *E.coli,* 16 for *K.pneumoniae*, 13 for *P.aeruginosa*, 26 for *S.aureus*, 53 for *M.luteus*, and 14 for *Ent.faecalis*. *M.luteus* inhibition rates are the highest of all pathogens for SGA and RSD. In the case of SGB, *E.coli* and *K.pneumoniae* have equal highest levels of inhibition. SGA and SGB have very similar activity levels across all pathogens, but SGA-derived isolates caused a higher proportion of inhibition at the '+++' level.

Positive results were collated and used to create pie charts showing the contribution of each sample type to overall activity (Figure 4). Based on these charts, contributions to overall activity were attributed 47.9% to SGA, 17.3% to SGB, and 34.8% to RSD. As different numbers of isolates from each sample type were tested, these proportions must be considered to compare activity levels (Table 5). When percentages of isolates tested from each sample type are factored in, the spread of activity is more even, with proportionate representation in terms of activity for each sample type, and no great difference between activity and testing contributions.

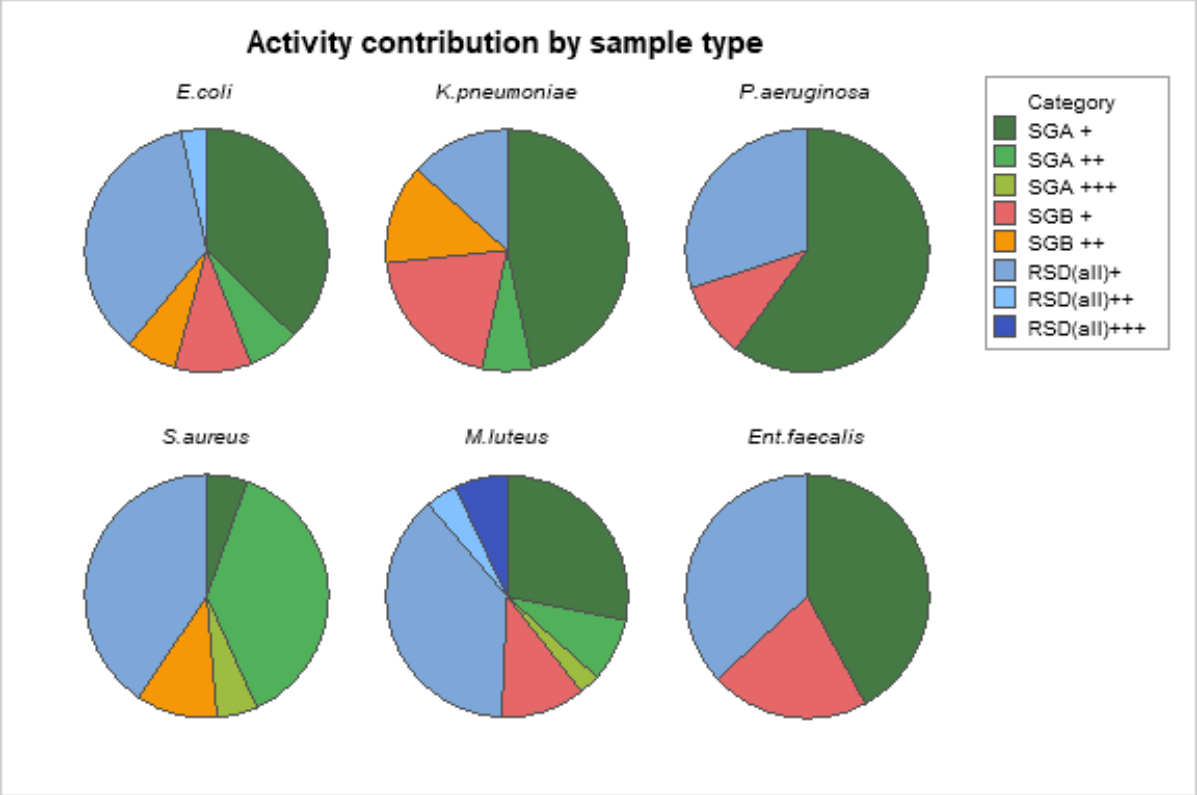
Table 5: Breakdown of isolates tested in simultaneous antagonism by sample type, and total isolate activity by sample type. Sample column shaded according to source: SGA in blue, SGB in orange, and RSD (combined) in purple.

Sample	Proportion of all isolates tested (%)	Proportion of total isolate activity (%)	Proportion of Gram positive activity (%)	Proportion of Gram-negative activity (%)
SGA	48.2	47.9	43.4	52.5
SGB	16.3	17.3	14.4	20.1
RSD	35.5	34.8	42.2	27.4



Note: The following criteria was used to assess activity:
'-' = No inhibition zone
'+' = Either no inhibition zone but growth of isolate around inoculation point, or small opaque zone (halo)
'++' = Medium to large size opaque zone, or small clear inhibition zone (with or without growth around inoculation point)
'+++' = Medium to large size clear inhibition zone with or without growth around inoculation point.

Figure 3 shows activity levels of isolates from (a) Seagrass A (SGA), (b) Seagrass B SGB, and (c) Root sediment (mean, RSD) in simultaneous antagonism assays against six pathogens.



Note: The following criteria was used to assess activity:
'-' = No inhibition zone.
'+' = Either no inhibition zone but growth of isolate around inoculation point, or small opaque zone (halo)
'++' = Medium to large size opaque zone, or small clear inhibition zone (with or without growth around inoculation point)
'+++' = Medium to large size clear inhibition zone with or without growth around inoculation point.

Figure 4: Contributions of each sample type and activity level towards overall activity against each pathogen in simultaneous antagonism tests. Seagrass A (SGA), Seagrass B SGB, and Root sediment (mean, RSD).

Cross streak

All 139 isolates from simultaneous antagonism testing were streaked on MHA. Of all 139, only 86 grew in a way which was suitable for cross streak testing (confluent line). Some had no growth, and some grew too quickly for testing. Of those tested, 30 were from MA(-NA), 29 were from R2A, 12 were from MA(+NA), and 15 were from ACT. 36 were from SGA, 11 were from SGB, 12 from RSD, and 27 from RSD(2). A '+' result was recorded if there was any evidence of pathogen growth inhibition, and '-' if there was not. An example can be seen in SF5. A total of 40 isolates had activity against one or more pathogen. Three isolates had activity against two pathogens (ECRSD(2)162, ECSGB188, ECRSD(2)204), and four isolates had activity against three (ECSGB60, ECRSD(2)115, ECSGA126, ECRSD(2)202). Of those active against three pathogens, two were active against all Gram positives tested, and two were active against *M.luteus, S.aureus*, and *E.coli*. Proportions of activity from each sample type were fairly even. *M.luteus* was most easily inhibited. In contrast, growth of *K.pneumoniae* and *P.aeruginosa* was not inhibited by any isolates in the cross-streak. In general, isolates had more activity against Gram positive pathogens than the Gram-negative pathogens tested.

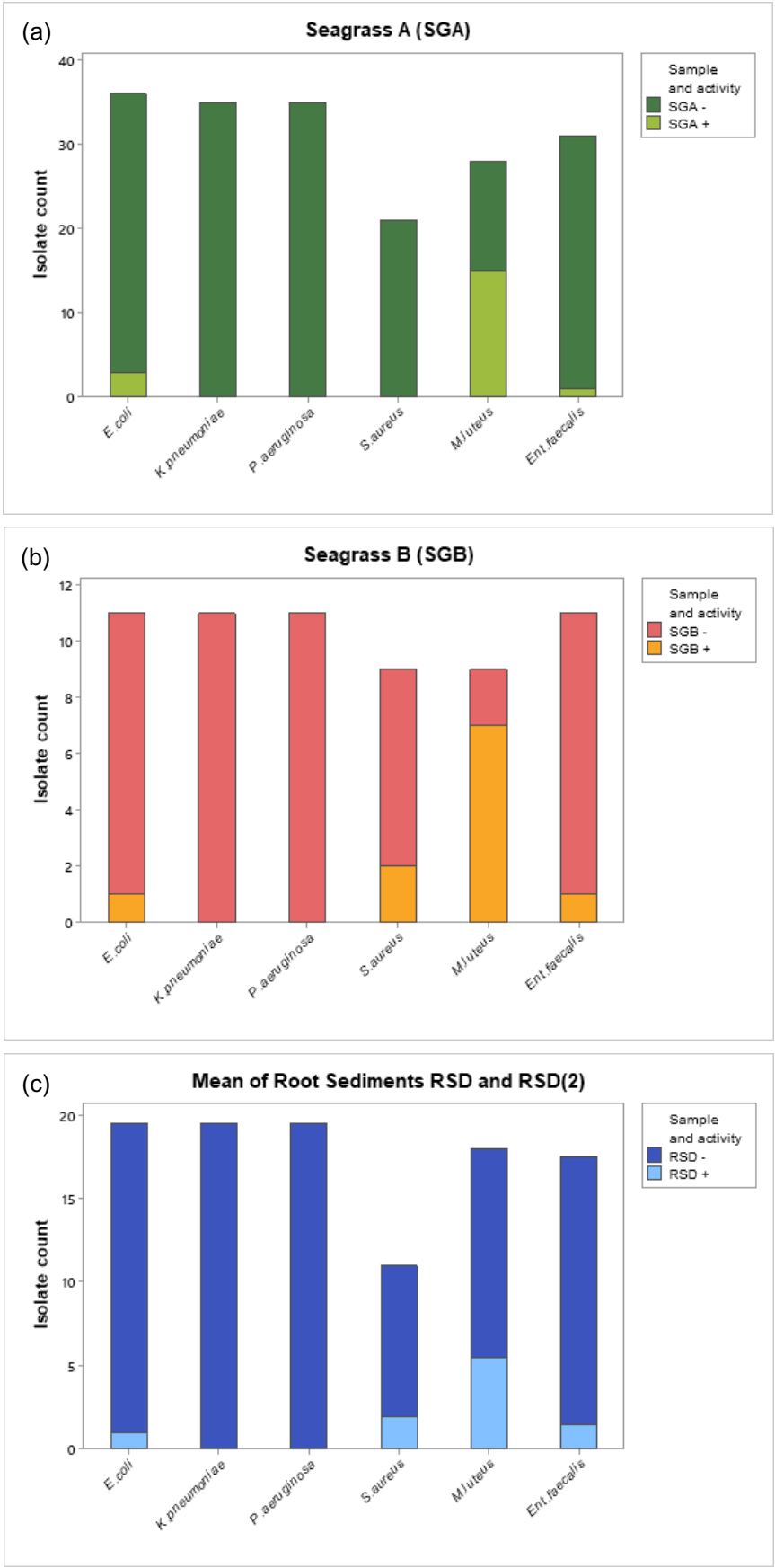


Figure 5: Cross-streak activity for all six pathogens for isolates tested in (a) Seagrass A (SGA), (b) Seagrass B (SGB), and (c) Root sediment mean (RSD and RSD(2)). The sample source followed by '+' represents inhibition, and '-' represents a lack of inhibition in that test.

Well diffusion
Well diffusion tests were performed on a narrowed down testing batch of 24, which included some isolates with activity in other tests. 7 isolates were from MA(-NA), 14 were from R2A, and 3 were from ACT. 9 Isolates came from SGA, 5 were from SGB, 3 from RSD, and 7 from RSD(2). For all six pathogens tested, there were no clear zones of inhibition. Three isolates produced feint halos when compared with control wells: ECSGB20 on *M.luteus*, ECSGB25 against *K.pneumoniae*, and ECRSD(2)115 against *Ent.faecalis*. Images are available in SF6.

Activity across multiple tests
31 isolates with activity on cross streaks also had activity on simultaneous antagonisms. Only one isolate had activity across all three tests ECSGB25. ECSGB25 had simultaneous antagonism activity against *E.coli* (++), cross streak activity against *M.luteus,* and well diffusion activity against *K.pneumoniae.* Data available in ST2, ST3, ST4.

Discussion
The discovery of bacterial isolates with activity against human pathogens provides potential for the development of new antibiotics to combat increasing AMR. This research aimed to address knowledge gaps surrounding the microbial composition, and potential antibacterial activity of isolates from, leaf- and root sediment-associated bacterial communities from a *Zostera marina* bed in Southwest England, an underexplored region. Differences in the diversity and composition of two seagrass microbial communities were ascertained, and bacterial isolate bioactivity potentials were established using 16S amplicon sequencing and culture-dependent techniques respectively.

Colony iridescence
Rainbow-diffuse iridescence was a property observed in some isolates, the majority of which came from seagrass leaf samples at a 20cm depth. This characteristic has been reported of marine bacteria previously, but there is a lack of information on this effect in isolates from the UK. The nearest known sources appear to be on the West coast of France, as described by Kientz *et al.,* 2012 and Kientz *et al.,* 2013. Iridescence is the result of structural coloration, and the periodic organisation of nanostructures to create a scattering of spectral colours (Schertel et al., 2020). Proposed biological roles in prokaryotes include UV protection, light filtering, and thermoregulation, and are hypothesised of marine bacteria derived from harsh rocky shore ecosystems (Kientz et al., 2013), applicable to Cellar's Beach as an estuarine rocky subtidal habitat. Findings of iridescence in leaf-, but not sediment-derived Yealm Estuary isolates may help to describe iridescence as a characteristic which serves to protect the seagrass leaves. This could be a relationship similar to that causing the distribution of nitrogen-fixing macroalgal bacteria as described by Goecke *et al.,* 2010. Potential evidence for this protective role can be seen in one study using bacterial isolates from sea mud in central Japan. Okazaki, Kithara and Okami, 1975 found that the bacteria *Chainia purpurogena* would only exhibit antibacterial activity when in the presence of its host-like growth conditions.

In previous studies, the Flavobacteriaceae family has been discussed in terms of colony iridescence, and *Cellulophaga lytica* is one species of particular interest (Kientz et al., 2012). It is possible that some of the isolates with iridescence such as

ECSGA44 and ECSGA98 belong to this family, particularly given that for seagrass A, Flavobacteriaceae was one of the top taxa found in 16S amplicon survey. It must be considered that other studies use specific angles to assess iridescence (Kientz et al., 2013), whereas there was no standardisation for such observations in this work. Furthermore, not all isolates were tested for iridescence. As it stands, iridescence of marine bacteria in relation to bioactivity has only been explored in one study, which described algicidal effects (Skerratt et al., 2002). In future research, a deeper exploration of this property and any host metabolic interactions may help to establish a potential selective advantage for the bacteria themselves, especially if a protective function for the host is established. This would be advantageous for natural product discovery, as dependencies on environmental or host factors for protective metabolite production can be exploited in laboratory testing to find new compounds.

Isolate identification

The sequencing of isolates ECSGA124 and ECRSD(2)202 revealed they both belong to the Actinobacteria phylum, known for encompassing Gram positive producers of biologically active molecules. The *Micromonospora* and *Streptomyces* genera identified are frequently isolated from marine samples and known for high levels of antimicrobial activity (Girão et al., 2019). Studies investigating different geographical locations describe the same dominance in sediment microbiomes and release of antimicrobial natural products, including samples from the Madeira Archipelago (Prieto-Davó et al., 2016) and the Trondheim Fjord in Norway (Bredholt et al., 2008). *Streptomyces* species are known producers of bioactive secondary metabolites. Example strains such as *Streptomyces griseorubens* f8 produce cyclic dipeptides with activity against *Staphylococcus aureus* (like isolate ECRSD(2)202) when assayed using filter paper discs in antibacterial susceptibility testing (Yang et al., 2021). Ikarugamycin is a polycyclic tetramic acid macrolactam sourced from *Streptomyces* with activity against MRSA. Structural derivatives of Ikarugamycin have been found from a marine species closely related to *Streptomyces zhaozhouensis*, and all have additional antibacterial potential (Lacret et al., 2014). Similarly, isolates belonging to the genus *Micromonospora* have produced many active macrolides including rosamicin, megalomicins, and mycinamicin (Hifnawy et al., 2020). The genera of these two sequenced, active, isolates therefore come as no surprise.

At a lower taxonomic rank, it must be considered that the stated identities of these isolates are derived from databases only containing other known species and strains. For both isolates, the top three BLASTn hits had the same % coverage and % identities so without further nucleic acid analysis or longer consensus sequences, their true identities (new or familiar) cannot be certain. What also remains to be established are the secondary metabolites giving the inhibition shown in cross streak and simultaneous antagonism testing. In further research, the identification of exact natural products from isolates ECSGA124 and ECRSD(2)202, using high-performance liquid chromatography, HPLC, and mass spectrometry for example (Lacret et al., 2014), would benefit establishing whether these are known or new compounds. This would give a clearer indication as to the true antibiotic potential of culturable bacteria from microbial communities in this location.

Seagrass leaf versus rhizosphere microbial diversity
A higher microbial diversity, measured using alpha diversity indices to ascertain species richness, was observed in the root-associated sediment versus the seagrass leaf at 20cm depth. This result is consistent with other findings including Fahimipour et al., 2017, which explored rhizospheric and phyllospheric microbial communities in a global study of *Zostera marina*. Similar observations are true, and more thoroughly explored, in terrestrial plants whose rhizobiomes have been further divided into three different niches, shaped by soil source and host genotypes (Edwards et al., 2015), factors also applicable to marine angiosperms (Schenck et al., 2023).

It is known that seagrasses and their microbial communities play important roles in ecological and biogeochemical cycles but are greatly impacted by chemical and physical soil disturbances (Bourque, Kenworthy and Fourqurean, 2015) as well as biotic factors such as plant metabolism (Fahimipour et al., 2017). Vessel grounding and propellor or anchor damage are particularly disruptive activities which cause seagrass bed fragmentation in areas where recreational boating is popular, such as the Yealm Estuary (South Devon AONB, 2020). These disturbances also reduce infaunal diversity, alter invertebrate communities, and result in the loss of soil which stores organic carbon, nitrogen, and phosphorous, and the microorganisms responsible for their cycling (Bourque, Kenworthy and Fourqurean, 2015). Correlations between the diversity of epibiotic eukaryotes and prokaryotic microbiomes (Bengtsson et al., 2017), indicate that disturbances to invertebrates will too impact microbial diversity. This emphasises the influences of abiotic factors in the dynamic microbial diversities of seagrass components, especially given this study's sampling location and its relevance to human stressors and current geographical gaps in profiling. Further microbiome characterisation alongside measurements of deviation from core taxa, and environmental parameters in this area would allow an increased understanding of the mechanisms by which this diversity is affected (Mejia et al., 2016).

Seagrass leaf versus rhizosphere microbial composition
Distinct microbial compositions across both seagrass components were also observed. Since a multitude of variables influence microbial communities, and factors such as geographical location have been shown to affect composition (Ettinger et al., 2017; Kardish and Stachowicz, 2023), it is difficult to narrow down the contribution of any one individual variable to the microbial habitats of a given seagrass plant. Microbial compositions of the samples are therefore better viewed as an aggregation of the influence of different factors over time. This is proven for the phyllosphere through studies comparing leaf age and its influence on the composition, abundance, and distribution of OTUs, with higher regional distinction of epiphytes in more mature leaves that have an increased history of habitat filtering (Sanders-Smith et al., 2020). Between different seagrass meadows, and within given geographical locations, there are also component-dependent microbial variations. On an even smaller scale, differences in the top sediment taxa have been observed according to individual sampling locations relative to the bed as a whole (Ettinger et al., 2017). This shows that local environmental factors such as nutrient availability, and subsequent plant metabolism have a greater impact on bacterial accumulation than species-specific factors alone (Cúcio et al., 2016).

Differences in core taxa of living seagrass components on this local scale are attributed to roles in environmental filtering, which differ in nature and strength, especially due to gradients of temperature, water flow, and the sediment organic content found in estuaries (Kardish and Stachowicz, 2023). Despite the subsequent effect on phyllospheric microbiomes, the spatial variance of these communities is far lower than for soil and sediment microbiomes, which are not subject to this filtering (Rabbani et al., 2021). An additional degree of functional-dependence has been reported in microbial habitats of seagrasses, reflected at a fundamental level with a richness of species needed for sulphide-oxidation and other biogeochemical capacities in the rhizome, and in the leaves, species with roles as free radical scavengers to mitigate damaging effects of oxidative stress found at higher light intensities (Rabbani et al., 2021). It must be noted that other laboratory group members' environmental samples (e.g., sea sponges, soil) were also included for analysis, so calculations of top ten taxa are inclusive of their associated communities. Exclusively analysing the seagrass leaf and sediment samples would not only improve the representation of the top taxa present, but allow true beta diversity descriptions. Further, specific microbiome characterisation down to species level, beyond the top ten taxa at each taxonomic rank and covering multiple sampling sites would allow a more comprehensive profile of community composition, reflecting functional differences and their impact on antibacterial activity.

Isolate activity

Isolates from root sediment had the highest activity levels in proportion to the total sediment isolates tested from this location (Figure 3c), which comes as no great surprise. Many studies including one by Kannan, Arumugam and Anantharaman (2010) have reported higher activity levels from rhizosphere-derived isolates, compared with other plant components. The high microbial diversity in this plant region perhaps explains this, as it provides a greater range of potential producer species, all of which have important roles in their ecosystems, and diverse metabolisms (Adnan et al., 2018).

Although activity levels in this study are promising overall, a true and more thorough investigation into secondary metabolite production would come from the exploration and laboratory manipulation of stressors which elicit their release *in situ*. This comes from the knowledge that secondary metabolites act as a protective mechanism in response to pressures such as bioactive metals like copper (Posacka, Semeniuk and Maldonado, 2019), which was considered for use this study but without time to investigate its impact. In addition, complex interactions between plant and endophyte metabolisms provide area for investigation as previously mentioned regarding the necessity of host-specific conditions for antibacterial compound release in some isolates (Okazaki, Kithara and Okami, 1975). These relationships between host and endophyte metabolism induction and biochemical transformations are described in more detail by Ludwig-Müller, (2015). Similarly, marine isolates and specifically those from the estuarine habitat in the Yealm Estuary, will have developed dependencies on local nutrient and environmental factors in order to grow in the first place. Unless these requirements are met in the laboratory, they are unable to grow and be tested for activity, and form part of the majority of bacteria which are unculturable (Rodrigues and de Carvalho, 2022). Essentially, an ideal combination for bacterial isolation and metabolite discovery would include molecular techniques alongside a diverse range of culture techniques and conditions.

Future implications

Despite this and other studies demonstrating the potential of marine-derived bacteria and secondary metabolites to decelerate AMR, the poor profitability of antibiotic development versus other drugs remains a significant barrier in their route to market. Networks for academic/industry collaborations and knowledge sharing provide short to medium term solutions for antibiotic development bottlenecks, and can advance AMR-corrective and preventative efforts. Ultimately, addressing funding issues in research and development, and creating strong partnerships of companies, such as Biotech companies in Europe combating AntiMicrobial resistance (BEAM) Alliance, will create a more powerful position from which to raise public and governmental awareness and backing of research (Miethke et al., 2021).

Further, more widespread microbiome profiling and the addition of any metagenomic results to databases will allow a more thorough understanding of composition and functional relationships. This will be aided by recent advances in machine learning methods which provide exciting potential to monitor community dynamics, and link them to environmental stressors, helping to contextualise secondary metabolite production (Hernández Medina et al., 2022).

Conclusions

A total of 207 bacterial isolates were subcultured and subsequently the antibacterial activity of pure colonies was tested. In simultaneous antagonism tests, 26 out of 139 isolates inhibited *Staphylococcus aureus*, and 41 inhibited *Escherichia coli*. Sediment-derived isolates had the most overall activity, including isolate growth and inhibition at the three assessed levels. In addition, the sediment microbiome showed higher alpha diversity, and highest operational taxonomic units (OTUs) of the *Bacillus* and *Sporosarcina* genera. These results are consistent with reports from similar studies using comparable samples. Predominant genera in the seagrass leaf from 20cm depth were *Marinomonas, Colwellia*, and *Winogradskyella.*

This work provides a contribution to what little knowledge there is of seagrass microbiomes, associated culturable bacteria, and their antibacterial activity, in Southwest England. It has also proved the potential for the discovery of novel natural products in these environmental samples and established marine bacteria in this location as an attractive and potentially fruitful area for research at the beginning of the antibiotic discovery pipeline. A foundation has also been provided for the characterisation of the secondary metabolites produced in activity tests. In addition, the environmental conditions for marine bacteria *in situ* have been discussed, providing options for their manipulation to increase bacterial culturability and elicit secondary metabolite production.

Acknowledgements

I would like to thank Professor Mathew Upton for his expertise and advice on planning and conducting this project and allowing me into his lab to do it.
This project would not have been possible without the help of other members of Mat's research group at Derriford: Grant, Jazz, Katie, and Michele, who were supportive, patient, and welcoming.

Thanks also to Richard Hartley and Robert Clough for their advice on water sampling and mass spectrometry, and to Jamie Quinn and Shaun Lewin from the GeoMapping unit for helping with maps and referencing.

References

Adnan, M., Alshammari, E., Patel, M., Amir Ashraf, S., Khan, S. and Hadi, S. (2018). Significance and potential of marine microbial natural bioactive compounds against biofilms/biofouling: necessity for green chemistry. *PeerJ*, 6, p.e5049.

Beal, J., Farny, N.G., Haddock-Angelli, T., Selvarajah, V., Baldwin, G.S., Buckley-Taylor, R., Gershater, M., Kiga, D., Marken, J., Sanchania, V., Sison, A. and Workman, C.T. (2020). Robust estimation of bacterial cell count from optical density. *Communications Biology*, [online] 3(1), pp.1–29. Available at: https://www.nature.com/articles/s42003-020-01127-5.

Bengtsson, M.M., Bühler, A., Brauer, A., Dahlke, S., Schubert, H. and Blindow, I. (2017). Eelgrass Leaf Surface Microbiomes Are Locally Variable and Highly Correlated with Epibiotic Eukaryotes. *Frontiers in Microbiology*, 8.

Bhatnagar, I. and Kim, S.-K. (2010). Immense Essence of Excellence: Marine Microbial Bioactive Compounds. *Marine Drugs*, [online] 8(10), pp.2673–2701. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2993000/ [Accessed 4 Nov. 2019].

Bourque, A., Kenworthy, W. and Fourqurean, J. (2015). Impacts of physical disturbance on ecosystem structure in subtropical seagrass meadows. *Marine Ecology Progress Series*, 540, pp.27–41.

Boyd, N.K., Teng, C. and Frei, C.R. (2021). Brief Overview of Approaches and Challenges in New Antibiotic Development: A Focus On Drug Repurposing. *Frontiers in Cellular and Infection Microbiology*, 11.

Bredholt, H., Fjærvik, E., Johnsen, G. and Zotchev, S. (2008). Actinomycetes from Sediments in the Trondheim Fjord, Norway: Diversity and Biological Activity. *Marine Drugs*, 6(1), pp.12–24.

Carvajal, F. (1947). Screening Tests for Antibiotics. *Mycologia*, 39(1), p.128.

Casella, T.M., Eparvier, V., Mandavid, H., Bendelac, A., Odonne, G., Dayan, L., Duplais, C., Espindola, L.S. and Stien, D. (2013). Antimicrobial and cytotoxic secondary metabolites from tropical leaf endophytes: Isolation of antibacterial agent pyrrocidine C from Lewia infectoria SNB-GTC2402. *Phytochemistry*, 96, pp.370–377.

Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S.J., Kubiszewski, I., Farber, S. and Turner, R.K. (2014). Changes in the global value of ecosystem services. *Global Environmental Change*, [online] 26(26), pp.152–158. Available at: https://www.sciencedirect.com/science/article/pii/S0959378014000685.

Cúcio, C., Engelen, A.H., Costa, R. and Muyzer, G. (2016). Rhizosphere Microbiomes of European + Seagrasses Are Selected by the Plant, But Are Not Species Specific. *Frontiers in Microbiology*, 7.

Dadgostar, P. (2019). Antimicrobial Resistance: Implications and Costs. *Infection and Drug Resistance*, [online] Volume 12(12), pp.3903–3910. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6929930/.

de Carvalho, C.C.C.R. and Fernandes, P. (2010). Production of Metabolites as Bacterial Responses to the Marine Environment. *Marine Drugs*, [online] 8(3), pp.705–727. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2857360/ [Accessed 6 Jan. 2022].

Dzobo, K. (2022). The Role of Natural Products as Sources of Therapeutic Agents for Innovative Drug Discovery. *Comprehensive Pharmacology*, [online] pp.408–422. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8016209/.

Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N.K., Bhatnagar, S., Eisen, J.A. and Sundaresan, V. (2015). Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences*, 112(8), pp.E911–E920.

Ettinger, C.L., Voerman, S.E., Lang, J.M., Stachowicz, J.J. and Eisen, J.A. (2017). Microbial communities in sediment from Zostera marina patches, but not the Z. marina leaf or root microbiomes, vary in relation to distance from patch edge. *PeerJ*, 5, p.e3246.

Fahimipour, A.K., Kardish, M.R., Lang, J.M., Green, J.L., Eisen, J.A. and Stachowicz, J.J. (2017). Global-Scale Structure of the Eelgrass Microbiome. *Applied and Environmental Microbiology*, 83(12).

Fischbach, M.A. and Walsh, C.T. (2009). Antibiotics for Emerging Pathogens. *Science*, 325(5944), pp.1089–1093.

Frank, J.A., Reich, C.I., Sharma, S., Weisbaum, J.S., Wilson, B.A. and Olsen, G.J. (2008). Critical Evaluation of Two Primers Commonly Used for Amplification of Bacterial 16S rRNA Genes. *Applied and Environmental Microbiology*, [online] 74(8), pp.2461–2470. doi:https://doi.org/10.1128/aem.02272-07.

Gagné, T.O., Reygondeau, G., Jenkins, C.N., Sexton, J.O., Bograd, S.J., Hazen, E.L. and Van Houtan, K.S. (2020). Towards a global understanding of the drivers of marine and terrestrial biodiversity. *PLOS ONE*, 15(2), p.e0228065.

Ghebretinsae, M., Adhanom, A., Ghebremedhin, H. and Tsegay, B. (2019). A REVIEW ON BIOACTIVE SECONDARY METABOLITES OF SEAGRASS OF THE SOUTHERN RED SEA, ERITREA. *International Journal of Advanced Research*, 7(3), pp.306–314.

Girão, M., Ribeiro, I., Ribeiro, T., Azevedo, I.C., Pereira, F., Urbatzka, R., Leão, P.N. and Carvalho, M.F. (2019). Actinobacteria Isolated From Laminaria ochroleuca: A Source of New Bioactive Compounds. *Frontiers in Microbiology*, 10.

Goecke, F., Labes, A., Wiese, J. and Imhoff, J. (2010). Chemical interactions between marine macroalgae and bacteria. *Marine Ecology Progress Series*, 409, pp.267–299.

Green, A., Chadwick, M.A. and Jones, P.J.S. (2018). Variability of UK seagrass sediment carbon: Implications for blue carbon estimates and marine conservation management. *PLOS ONE*, 13(9), p.e0204431.

Hernández Medina, R., Kutuzova, S., Nielsen, K.N., Johansen, J., Hansen, L.H., Nielsen, M. and Rasmussen, S. (2022). Machine learning and deep learning applications in microbiome research. *ISME Communications*, 2(1).

Hifnawy, M.S., Fouda, M.M., Sayed, A.M., Mohammed, R., Hassan, H.M., AbouZid, S.F., Rateb, M.E., Keller, A., Adamek, M., Ziemert, N. and Abdelmohsen, U.R. (2020). The genus Micromonospora as a model microorganism for bioactive natural product discovery. *RSC Advances*, 10(35), pp.20939–20959.

Kannan, R.R.R., Arumugam, R. and Anantharaman, P. (2010). Antibacterial potential of three seagrasses against human pathogens. *Asian Pacific Journal of Tropical Medicine*, 3(11), pp.890–893.

Kardish, M.R. and Stachowicz, John.J. (2023). Local environment drives rapid shifts in composition and phylogenetic clustering of seagrass microbiomes. *Scientific Reports*, 13(1).

Kharlamenko, V., Kiyashko, S., Imbs, A. and Vyshkvartzev, D. (2001). Identification of food sources of invertebrates from the seagrass Zostera marina community using carbon and sulfur stable isotope ratio and fatty acid analyses. *Marine Ecology Progress Series*, 220, pp.103–117.

Kientz, B., Agogué, H., Lavergne, C., Marié, P. and Rosenfeld, E. (2013). Isolation and distribution of iridescent Cellulophaga and other iridescent marine bacteria from the Charente-Maritime coast, French Atlantic. *Systematic and Applied Microbiology*, 36(4), pp.244–251.

Kientz, B., Vukusic, P., Luke, S. and Rosenfeld, E. (2012). Iridescence of a Marine Bacterium and Classification of Prokaryotic Structural Colors. *Applied and Environmental Microbiology*, 78(7), pp.2092–2099.

Lacret, R., Oves-Costales, D., Gómez, C., Díaz, C., de la Cruz, M., Pérez-Victoria, I., Vicente, F., Genilloud, O. and Reyes, F. (2014). New Ikarugamycin Derivatives with Antifungal and Antibacterial Properties from Streptomyces zhaozhouensis. *Marine Drugs*, 13(1), pp.128–140.

Lilley, R.J. and Unsworth, R.K.F. (2014). Atlantic Cod (Gadus morhua) benefits from the availability of seagrass (Zostera marina) nursery habitat. *Global Ecology and Conservation*, 2, pp.367–377.

Ludwig-Müller, J. (2015). Plants and endophytes: equal partners in secondary metabolite production? *Biotechnology Letters*, 37(7), pp.1325–1334.

Mejia, A.Y., Rotini, A., Lacasella, F., Bookman, R., Thaller, M.C., Shem-Tov, R., Winters, G. and Migliore, L. (2016). Assessing the ecological status of seagrasses using morphology, biochemical descriptors and microbial community analyses. A study in Halophila stipulacea (Forsk.) Aschers meadows in the northern Red Sea. *Ecological Indicators*, 60, pp.1150–1163.

Miethke, M., Pieroni, M., Weber, T., Brönstrup, M., Hammann, P., Halby, L., Arimondo, P.B., Glaser, P., Aigle, B., Bode, H.B., Moreira, R., Li, Y., Luzhetskyy, A., Medema, M.H., Pernodet, J.-L., Stadler, M., Tormo, J.R., Genilloud, O., Truman, A.W. and Weissman, K.J. (2021). Towards the sustainable discovery and development of new antibiotics. *Nature Reviews. Chemistry*, [online] 5, pp.1–24. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8374425/?log.

Mills, S.D. (2006). When will the genomics investment pay off for antibacterial discovery? *Biochemical Pharmacology*, 71(7), pp.1096–1102.

Murray, C.J., Ikuta, K.S., Sharara, F., Swetschinski, L., Aguilar, G.R., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S.C., Browne, A.J., Chipeta, M.G., Fell, F., Hackett, S., Haines-Woodhouse, G., Hamadani, B.H.K., Kumaran, E.A.P., McManigal, B. and Agarwal, R. (2022). Global Burden of Bacterial Antimicrobial Resistance in 2019: A Systematic Analysis. *The Lancet*, [online] 399(10325), pp.629–655. Available at: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(21)02724-0/fulltext.

O'Neill, J. (2016). *TACKLING DRUG-RESISTANT INFECTIONS GLOBALLY: FINAL REPORT AND RECOMMENDATIONS*. [online] Available at: https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf [Accessed 20 Feb. 2023].

Okazaki, T., Kithara, T. and Okami, Y. (1975). Studies on marine microorganisms. IV. A new antibiotic SS-228 Y produced by Chainia isolated from shallow sea mud. *The Journal of Antibiotics*, 28(3), pp.176–184.

Olsen, J.L., Rouzé, P., Verhelst, B., Lin, Y.-C., Bayer, T., Collen, J., Dattolo, E., De Paoli, E., Dittami, S., Maumus, F., Michel, G., Kersting, A., Lauritano, C., Lohaus, R., Töpel, M., Tonon, T., Vanneste, K., Amirebrahimi, M., Brakel, J. and Boström, C. (2016). The genome of the seagrass Zostera marina reveals angiosperm adaptation to the sea. *Nature*, [online] 530(7590), pp.331–335. Available at: https://www.nature.com/articles/nature16548 [Accessed 21 Jan. 2022].

Posacka, A.M., Semeniuk, D.M. and Maldonado, M.T. (2019). Effects of Copper Availability on the Physiology of Marine Heterotrophic Bacteria. *Frontiers in Marine Science*, 5.

Prieto-Davó, A., Dias, T., Gomes, S.E., Rodrigues, S., Parera-Valadez, Y., Borralho, P.M., Pereira, F., Rodrigues, C.M.P., Santos-Sanches, I. and Gaudêncio, S.P. (2016). The Madeira Archipelago As a Significant Source of Marine-Derived Actinomycete Diversity with Anticancer and Antimicrobial Potential. *Frontiers in Microbiology*, 7.

QIAGEN (2021). *DNeasy PowerSoil Pro Kit Handbook - QIAGEN*. [online] www.qiagen.com. Available at: https://www.qiagen.com/gb/resources/resourcedetail?id=9bb59b74-e493-4aeb-b6c1-f660852e8d97&lang=en [Accessed 22 Mar. 2023].

Rabbani, G., Yan, B.C., Lee, N.L.Y., Ooi, J.L.S., Lee, J.N., Huang, D. and Wainwright, B.J. (2021). Spatial and Structural Factors Shape Seagrass-Associated Bacterial Communities in Singapore and Peninsular Malaysia. *Frontiers in Marine Science*, 8.

Reidenbach, M.A. and Thomas, E.L. (2018). Influence of the Seagrass, Zostera marina, on Wave Attenuation and Bed Shear Stress Within a Shallow Coastal Bay. *Frontiers in Marine Science*, 5.

Rodrigues, C.J.C. and de Carvalho, C.C.C.R. (2022). Cultivating marine bacteria under laboratory conditions: Overcoming the 'unculturable' dogma. *Frontiers in Bioengineering and Biotechnology*, 10.

Sanders-Smith, R., Segovia, B.T., Forbes, C., Hessing-Lewis, M., Morien, E., Lemay, M.A., O'Connor, M.I. and Parfrey, L.W. (2020). Host-Specificity and Core Taxa of Seagrass Leaf Microbiome Identified Across Tissue Age and Geographical Regions. *Frontiers in Ecology and Evolution*, 8.

Schenck, F.R., DuBois, K., Kardish, M.R., Stachowicz, J.J. and Hughes, A.R. (2023). The effect of warming on seagrass wasting disease depends on host genotypic identity and diversity. *Ecology*.

Schertel, L., van de Kerkhof, G.T., Jacucci, G., Catón, L., Ogawa, Y., Wilts, B.D., Ingham, C.J., Vignolini, S. and Johansen, V.E. (2020). Complex photonic response reveals three-dimensional self-organization of structural coloured bacterial colonies. *Journal of The Royal Society Interface*, 17(166), p.20200196.

Shang, J., Hu, B., Wang, J., Zhu, F., Kang, Y., Li, D., Sun, H., Kong, D.-X. and Hou, T. (2018). Cheminformatic Insight into the Differences between Terrestrial and Marine Originated Natural Products. *Journal of Chemical Information and Modeling*, 58(6), pp.1182–1193.

Skerratt, J., Bowman, J., Hallegraeff, G., James, S. and Nichols, P. (2002). Algicidal bacteria associated with blooms of a toxic dinoflagellate in a temperate Australian estuary. *Marine Ecology Progress Series*, 244, pp.1–15.

Smith, A. and Hussey, M. (2005). *Gram Stain Protocols*. [online] *American Society for Microbiology*. Available at: https://asm.org/getattachment/5c95a063-326b-4b2f-98ce-001de9a5ece3/gram-stain-protocol-2886.pdf.

South Devon AONB (2020). *Seagrass beds of the Yealm Estuary*. [online] South Devon AONB. Available at: https://www.southdevonaonb.org.uk/wp-content/uploads/2020/01/Seagrass_Beds_of_the_Yealm_Estuary_leaflet.pdf [Accessed 20 Mar. 2023].

Srinivasan, R., Kannappan, A., Shi, C. and Lin, X. (2021). Marine Bacterial Secondary Metabolites: A Treasure House for Structurally Unique and Effective Antimicrobial Compounds. *Marine Drugs*, 19(10), p.530.

Sun, F., Zhang, X., Zhang, Q., Liu, F., Zhang, J. and Gong, J. (2015). Seagrass (Zostera marina) Colonization Promotes the Accumulation of Diazotrophic Bacteria and Alters the Relative Abundances of Specific Bacterial Lineages Involved in Benthic Carbon and Sulfur Cycling. *Applied and Environmental Microbiology*, 81(19), pp.6901–6914.

Tagg, J.R. and Bannister, L.V. (1979). 'Fingerprinting' -haemolytic streptococci by their production of and sensitivity to bacteriocine-like inhibitors. *Journal of Medical Microbiology*, 12(4), pp.397–411.

Ugarelli, K., Chakrabarti, S., Laas, P. and Stingl, U. (2017). The Seagrass Holobiont and Its Microbiome. *Microorganisms*, 5(4), p.81.

Valgas, C., Souza, S.M. de, Smânia, E.F.A. and Smânia Jr., A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*, [online] 38(2), pp.369–380. Available at: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1517-83822007000200034.

Wang, L., English, M.K., Tomas, F. and Mueller, R.S. (2020). Recovery and Community Succession of the Zostera marina Rhizobiome after Transplantation. *Applied and Environmental Microbiology*, 87(3).

Yang, W., Liang, G., Sun, Y. and Gong, Z. (2021). Bioactive Secondary Metabolites from Marine Streptomyces griseorubens f8: Isolation, Identification and Biological Activity Assay. *Journal of Marine Science and Engineering*, 9(9), p.978.

Appendices are provided separately as supplementary files (see additional downloads for this article).