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Deciphering the seagrass microbiome for advanced ecosystem restoration

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Abstract

Seagrasses are important stores of blue carbon and provide important ecosystem services such supporting commercially important fish populations and reducing effects of pollution and erosion; however, seagrasses are in decline because of anthropogenic activities and climate change. Like terrestrial plants, seagrasses depend on mutualistic relationships with bacteria that are central to their health. The purpose of this study was to investigate the effect of bacteria on seagrass germination with the overall prospect of utilizing them as probiotics for seagrass restoration.

Seagrass seeds and sediment samples were collected from a restoration nursery in Carmarthenshire, UK. Bacterial strains were isolated from the seeds and sediment using a combination of marine broth and F/2-based growth media to capture diverse taxa. Representative colonies were restreaked, and identified by 16S rRNA gene sequencing, then preserved as cryostocks for subsequent experiments.

A laboratory-based germination assay was established to test the effects of different substrates (sediment, agar, and sterile seawater) on germination success. Of the three media, agar yielded the highest germination at 36.1% ($p = 0.3402$). Co-cultivation experiments demonstrated that some bacterial isolates reduced seed mortality, accelerated germination, and promoted seedling growth. For example, *Variovorax paradoxus* treatments resulted in higher cotyledon length (15 mm ± 7 mm) compared to the control (14.3 mm ± 8.7 mm). However, the results of statistical analysis did not yield a significance in these effects.

Furthermore, bioinformatic analyses were utilized to identify relevant pathways in the bacterial genomes including secondary metabolites to further understand the relationship they may have with seagrass.

The four analyzed bacteria genomes were found to encode for enzymes that break down cell wall components, which are vital for cycling nutrients that are important for seagrass growth. Biosynthetic gene clusters for secondary metabolites were identified in the genomes that have functions in antifouling, and communication (terpenes) that positively influence seagrass health and growth. This study shows the importance of seagrass associated bacteria to health and growth of seedlings and adult plants. Furthermore, it provides evidence that strains such as *V. paradoxus* can be used in biofertilizers to enhance conservation of the seagrass ecosystem.

Lay abstract

Seagrasses are marine plants that play a critical role in the environment, primarily by storing blue carbon, which refers to the carbon captured by marine ecosystems. This is important in the fight against climate change because seagrasses absorb carbon dioxide from the atmosphere. Additionally, seagrasses provide other key ecosystem services, such as supporting commercially valuable fish species, filtering pollutants, and reducing coastal erosion by stabilizing sediments. Seagrasses globally are declining due to anthropogenic activities like coastal development and pollution, as well as the broader impacts of climate change, such as rising sea temperatures. Seagrasses also have mutually beneficial relationships with bacteria, forming a microbiome that can help with nutrient uptake, disease resistance, and growth. However, the role these bacteria play, particularly in seed germination and plant growth, is not well understood. The goal of this study was to investigate whether bacterial strains isolated from seagrass seeds and surrounding sediments could influence seagrass seed germination and growth.

Initial experiments determined the optimal conditions for seagrass seed germination in controlled laboratory conditions. Three different growth mediums were tested: sediment, agar, and sterile seawater. Out of the three growth mediums, agar yielded the highest germination rate, at 36.1%. Following this, co-cultivation of seeds with different bacterial strains was done to determine if there would be an improvement of growth. The results showed that certain bacterial strains may decrease seed mortality, speed up germination, and increase seedling growth. For example, the strain *Variovorax paradoxus* increased the cotyledon length of seedlings, showing a positive impact on plant development.

In addition to the germination experiments, bioinformatic analyses on the genomes of four bacterial strains were performed. This was done to identify enzymes that break down plant cell wall components, which are important for nutrient cycling, providing essential nutrients for seagrass growth. The bacteria also were also analysed to produce secondary metabolites, which could play a role in antifouling (preventing pathogens from inhibiting growth) and enhancing communication between the plants and microbes

The findings of this study highlight the potential for certain bacterial strains, like *V. paradoxus*, to improve the health and growth of seagrass seedlings. This suggests that such bacteria could be used in biofertilizers, not only to enhance seagrass restoration efforts but also potentially to boost the growth of agricultural plants. In conclusion, this research underscores the importance of seagrass-associated bacteria in promoting plant health and offers new possibilities for conservation and restoration of seagrasses.

Declarations

This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

Sign [redacted].....

Date.....29/06/2025.....

This thesis is the result of my own investigations, except where otherwise stated. Other sources are acknowledged by footnotes giving explicit references. A bibliography is appended.

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I hereby give consent for my thesis, if accepted, to be available for electronic sharing

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Contents

0. List of tables and figures

Abbreviations

Abbreviation	Definition
AA	Auxiliary activities
CAZyme	Carbohydrate active enzymes
CE	Carbohydrate esterases
CFU	Colony forming units
dbCAN	Database for Carbohydrate-active enzymes
DNA	Deoxyribonucleic acid
GH	Glycoside hydrolases
GRt	Germination percentage
GT	Glycosyl transferases
IAA	Indole-3-acetic acid
PCR	Polymerase Chain Reaction
PL	polysaccharide lyases
RNA	Ribonucleic acid
Sd	Sediment

1. Introduction

Seagrasses are angiosperms that grow in shallow marine waters in all continents except Antarctica (Schubert and Demes, 2017). They occupy between 300 and 600km2 globally, acting as a blue carbon store for around 20% of the carbon dioxide absorbed by the sea (Fourqurean et al., 2012; Zhou et al., 2024). They also act as a food source for endangered megafauna such as dugongs, manatees and turtles, and provide nursery grounds for commercially important species of fish (Ugarelli et al., 2017; Gullström et al., 2002). Global warming and anthropogenic activities pose a threat to the health of seagrass ecosystems, as coastal development, nutrient runoff and increasingly polluted oceans are primary drivers of the global decline of seagrasses (Ren et al., 2024). This highlights the importance of restoration efforts globally. The research on seagrass conservation is well studied, with guidelines for preservation and restoration being laid out by government funded organizations (Unsworth et al., 2024). Additionally, seagrasses play important roles in the cycling of sulphur, nitrogen and carbon. These are processes which occur mostly in rhizomes and are mediated by bacteria that make up the rhizosphere (Martin et al., 2018; Donnelly and Herbert, 1998) to promote the cycling of these nutrients into the ecosystem.

The seagrass microbiome has many functions that contribute to the health and growth of the seagrass ecosystem (Sun et al., 2024). Numerous studies show statistical differences between the microbiome of the root of the plant, and the shoots and leaves. Bacterial communities around the roots are ten times more efficient at fixing nitrogen than bacterial communities around the leaves (Randell et al., 2023). Some seagrass leaf associated genera have also been documented, for example Methylophaga and Methylobacter, can break down harmful methanol that is produced as a byproduct of cell wall synthesis (Tarquinio et al., 2021). Pathogens can be neutralized by bacteria, such as Pseudoalteromonas *fuliginea* and Vreelandella titanicae, as antibacterial, antimicrobial and antioxidant metabolites that are produced, limit the effects of pathogens on the seagrass, and the ecosystem services they provide (Tarquinio et al., 2019; Wang et al., 2014). The seagrass microbiome is less understood than that of corals and sponges (Bourne et al., 2016; Webster and Thomas, 2016), but it is a growing field, meaning the functions that support fitness, growth, defence and germination are

becoming better understood, with important implications for the conservation and growth of the ecosystem (Ugarelli et al., 2017).

The focus is this study is to further understand the role of bacteria that make up the seagrass seed microbiome, and to determine whether individual species of bacteria have an impact on germination; this is a novel experimental design, as no studies have investigated these in such a controlled manner. Few studies have been done to investigate the effects of altering the microbiome on germination success. Typically, germination rates are around 20% in natural environments (Liu et al., 2023a). Studies have shown the manipulating the microbiome can have effects on growth and health of adult plants and seedlings. For example, the reduction of the microbiome by exposing seagrass to a weak bleach solution or antibiotics, can reduce the effects of saprotrophic parasites such as Labrynthula zosterae, as the pathogen feeds off the microbiome to infect the seagrass plant (Graham et al., 2024). This is a notable finding, as the pathogen, *L.* zosterae causes seagrass wasting disease, which has caused drastic declines to seagrass habitats. *L. zosterae* remains a threat as global warming and climate change weaken the resistance of seagrasses to these threats (Aoki et al., 2023).

Therefore, the aim of this study was to analyse the seagrass associated bacteria using bioinformatics and investigate whether isolated species of bacteria from the microbiome can impact seagrass seed germination. In agriculture, strains of rhizosphere associated bacteria have been documented to improve growth in crops (Schmalenberger et al., 2008). Hence, it was hypothesized that select strains will have a positive impact on germination success.

Gene sequencing of the 16S RNA gene was used to identify the species of bacteria present after isolating them from seed and sediment samples. The 16S RNA gene is a small ribosomal subunit that has been well documented as an ideal genetic marker for understanding phylogenetic relationships between celled organisms, providing reliable insights into bacterial taxonomy and diversity due to its highly conserved nature across different bacterial species (Medina-Pons et al., 2009). However, further analysis such as full genome sequencing, or amplified rDNA restriction analysis (Weidner et al., 1996) is required to obtain a more accurate phylogenetic identification of isolated strains.

Therefore, this study also utilized bioinformatics to reveal metabolic features of the full genome of four isolated strains of bacteria. This was done to find secondary metabolites and Carbohydrate active enzymes that may interact with seagrasses. Further research is needed to determine the architecture of seagrass cell walls (Pfeifer et al., 2022), but major components of the structures are known to be comprised of mostly Xylan's (38%) (Pfeifer and Classen, 2020), cellulose, lignin and pectin, along with other polysaccharides with various functional roles (Pfeifer et al., 2022; Olsen et al., 2016; Sugiura et al., 2009). The study will build on the body of information about the relationship of the seagrass microbiome and will aid in restoration efforts to the seagrass ecosystem.

2. Materials + Methods

2.1 Seagrass seeds and sediment samples

Seagrass seeds samples and sediment cores were collected from the Project Seagrass (Nursery - Project Seagrass, 2024) nursery in Carmarthenshire. The nursery began in 2020 with a goal to grow mature seagrass plants to support restoration efforts. Semi-controlled conditions aimed to increase the survival of seedlings. These Seedlings grew successfully into mature, flowering plants and even produced seeds. The facility also holds seeds that can be planted around the UK for restoration efforts. They held 1.2 million seeds in 2023, in conditions designed to minimize germination.

Seeds and sediment samples were collected for bacterial isolation on the 14th of March 2024 and stored at 5°C until the bacteria were isolated from the seeds on 15th of March 2024. Sediment cores were taken from three growing ponds:

- Sample 1 (Sd1): Plants were initially sown in March 2023 in a polytunnel pond into a sediment mix comprising 80% Kettering loam and 20% sand. In September 2023, these plants were transferred, along with their associated sediment, to outdoor ponds. No fertilizer was applied. This sediment sample was collected from the outdoor pond of the Seagrass nursery

- Sample 2 (Sd2): This sediment originated from natural sediment. Plants were first sown into this natural sediment in March 2022 within the polytunnel. In

November 2022, they were re-potted into fresh natural sediment of the same type, but kept indoors. No fertilizers were added at any stage. The sample was isolated from this re-potted material.

- Sample 3 (Sd3): Plants were sown into a mix of Kettering loam (80%) and sand (20%) in May 2022, re-potted in November 2022, and fed with Osmocot fertilizer in March 2023. The sediment was sampled from this pond to sample nutrient rich sediment.

The seeds used in this study were collected from wild seagrass meadows in north Wales. They were stored in darkness, aerated with a bubbler, at a salinity of 50 ppt and at a temperature of >5°C. These conditions limited germination and minimized the seeds from going bad. They were initially collected on the 26th of September 2023.

Prior to the preliminary and co-cultivation experiments, more seeds were delivered to the lab so they could be stored in the above conditions until their respective trials began on the following dates

- Preliminary trial: 29th April 2024 – 20th March 2024
- Co-cultivation trial 1: 27th May 2024 – 17th June 2024
- Co-cultivation trial 2: 25th June 2024 – 16th July 2024

2.2 Bacterial isolation

To isolate bacteria from seeds and sediment samples, three seeds were randomly selected and placed into an Eppendorf tube with 1 ml of sterile seawater. The seeds were vortexed for one minute to release the bacteria. 1 gram of each sediment sample was mixed into 1 ml of sterile seawater, vortexed, and then centrifuged at 2000*g to pellet the sediment. A tenfold dilution series to a dilution of 10^{-6} was performed to estimate the number of bacteria obtained from both sample types.

0.1 ml of each dilution from each seed and sediment sample was spread onto two types of agar-based growth media. Samples were inoculated on two types of media as bacteria with different functions require different nutrients to grow (Cifuentes et al., 2003; Prasad et al., 2018),

therefore, to isolate a variety of bacteria, marine broth media (as used by Prasad et al., 2018) and F/2 solution supplemented with ammonium sulphate and sucrose (as used by Cifuentes et al., 2003) (Table 1). Cultures were incubated at 25°C for 2 days, then the number of colonies was counted.

30 colonies were selected based on morphology and colour for each of the sediment and seed bacterial isolates. This method was adapted from a method by Sespian et al., 2018. They were re-streaked onto the media they were initially grown on, and in the same conditions, at 25°C. Of the 60 selected, 18 successfully grew again, and were cryogenically frozen in a 30% glycerol solution and isolation medium. Cryo-stocks were stored at –80°C for future use.

Table 1, Measurements for growth media components, with suppliers.

Marine broth media (per 100ml)	**F/2+Sucrose+Ammonium sulphate (per 100ml)**
MB/10 (Millipore), 0.37g	F/2 Solution (Sigma-Aldrich), 2ml
Agar (Formedium)1.5g	Ammonium sulphate (FlukaTM) 0.07g
Milli-Q water, 100ml	Sucrose (Sigma-Aldrich), 0.17g
	Seawater, 99ml

2.3 Colony counting

To estimate the number of bacteria, present in the sediment and seed samples, the following equation was used to calculate the number of colony forming units (CFU) present in either 1ml of sterile seawater from the seed samples, or 1g of sediment.

$$CFU/unit = Cn / Vn$$

Cn = The number of colonies forming units present

Vn = the dilution factor in which the colonies (Cn) were counted.

An average of 3 repeats were used for plates yielding CFU's within the countable range (30–300 colonies) (Tennant and Rutten, 2019). This approach helps ensure accuracy and consistency in the estimation of bacterial counts. This data was presented as a box plot using RStudio (RStudio Team, 2023) and ggplot2 (Wickham, 2016). T-tests were utilized to determine whether the mean CFU counts were different when grown on each media, for both sediment samples, and seed samples.

2.4 Taxonomic classification of bacterial isolates based on the 16S rRNA gene

To obtain 16s RNA gene sequencing, isolated bacterial strains were individually lysed in 50 µl of dH20 at 95°C for 5 mins. The lysate was then used as DNA template for PCR by mixing with 12.5 µl, 2x DreamTaq Green Master Mix, and 0.5 µl of both 27F (16s forward: AGAGTTTGATCMTGGCTCAG) and 1492R (16s reverse: TACGGYTACCTTGTTACGACTT) primer sets (Frank et al., 2008) (100 pmol/µl = µM), targeting approximately 1,500 bp and covering the V1–V9 variable regions.

PCR Amplification Conditions:

Initial denaturation of DNA: 95°C for 1:00 minute

Amplification (35 cycles)

Denaturation of DNA: 95°C for 0:30 minutes

Primer annealing temperature: 53°C for 0:30 minutes

Extension: 72°C for 1:00 minute

Final extension (1x): 72°C for 5:00 minutes

Hold: 8°C

The post PCR products were then run on a 1% agarose gel, to check if the PCR was successful. The PCR products were then purified using the GeneJET PCR purification kit (GeneJET PCR Purification Kit, 2024), before being sent off to Eurofins for Sanger sequencing.

The 16S rRNA gene sequences were then compared to the 16S rRNA ribosomal database, limited to type material and optimized for megablast, from blastN (NCBI, 2006). Sequences were aligned using Clustal X (Dineen, 2019), with the top three most homologous strains retrieved from blastN (NCBI, 2025). A Phylogenetic tree was constructed using the Neighbour- Joining, distance-based method. The strains were evaluated based on 1000 bootstrap replicates. The consensus tree was produced using MEGA (Molecular Evolutionary Genetics Analysis, 2019).

2.5. Preliminary germination experiment

To test the effects of different substrates on germination, a total of 90 seeds were placed into microwell plates (5 seeds per well, six replicate wells per treatment, for repeatability) with either 12 ml sterile sea water, 6 ml of 1% agar and 6 ml sterile seawater, or 5 g sterile sediment and 7 ml sterile seawater. The samples were incubated at 10°C, on a 12-hour light cycle at 3000 lumens for 21 days. The number of seeds germinated were recorded each day. Cotyledons were counted, and their length was measured, and mortality of seeds was recorded at the end of the experiment. Mortality is defined as a seed that has stopped growing, and significant browning of the cotyledon has occurred (Govers et al., 2017). Seeds with a cotyledon emerging through a split open seed were recorded as germinated (Churchill, 1983). Seeds were only considered alive at the end of the experiment if the cotyledons were either white or green. Brown cotyledons were recorded as deceased.

The Fishers exact test was used to determine if the different growth media (Seawater, Agar, and Sediment) yielded significant differences in germination by assessing overall independence between treatment and mean germination success, rather than pairwise comparisons. A post hoc test was then used to investigate differences in pairwise comparisons. This was done using R studio, and the ggplot2 package (Wickham, 2016) was used to visualize this data as a bar plot.

2.6. Co-cultivation germination

To study the effect of bacterial isolates on germination, 18 bacterial strains were grown in liquid media equivalent to the media they were initially grown on (see Appendix Table 5), for 2 days, at 25°C and shaking at 100rpm. Cultures were then diluted to an optical density of 0.1

(assuming an approximate cell concentration of 10^{-7} CFU/mL), measured by a spectrophotometer at 600 nm, and making measurements based off a calibration curve, as opposed to using drop counts (Stevenson et al., 2016). Five seeds were placed per well of a six well plate with 5mls of 1% agar, 7 ml of sterile seawater, and 700µl of the bacterial solution in four replicates (except for the negative control, where 4 wells had only agar and sterile seawater). Wells were incubated at 10°C with a 12-hour light cycle, for 21 days, at 3000 lumens (lm). The number of seeds germinated was recorded daily, Cotilions were counted and measured, and mortality of seeds were recorded at the end of the trial.

This experiment was repeated twice for strains 1-9 (Appendix Table 5) from 7th May 2024 – 17th June 2024, then with strains 9-18 (Appendix Table 5) from 25th June 2024 – 16th July 2024. However, the second experiment was less successful due to the advanced age of the seeds.

A bar plot was used to visualize the mean cumulative germination of the preliminary experiment using the following equation for germination percentage (GRt), as used by Liu et al., 2016.

GRt = nt/N*100% (nt) = cumulative number of seeds on a germination day

(N) = number of seeds in each well

The Ggplot2 (Wickham, 2016) package visualized the co-cultivation experiment, specifically the mean cotyledon length, time to germinate, and the mortality at the end of the experiments, with bars of standard deviation. Kruskal Wallace tests were used to determine if the different bacteria (Appendix Table 5) yielded significant differences in germination by assessing overall independence between treatment and mean germination success, rather than pairwise comparisons.

2.7. Bioinformatic analysis of bacterial genomes

To obtain complete bacterial genome sequences, DNA was extracted and purified from four selected strains (table 2), using the Promega extraction kit (Wizard® HMW DNA Extraction Kit, 2024). Concentrations were lower than the minimum of 20 ng/µl needed for Nanopore sequencing. Therefore, representative genomes were selected from the NCBI database based on closest similarity to the 16S rRNA gene of the bacterial isolates (Table 3).

Table 2. Selected strains for analysis of bacterial genomes: strain selection based on literature search. *P. fuliginea* is known for degradation and nutrient cycling. *Vreelandella titanicae* (Du et al., 2022) and *Psychromonas arctica* (Groudieva et al., 2003) known for cold resistance, and *Variovorax paradoxus* forms symbiotic relationships with plants (Han et al., 2011).

ID of bacterial isolate	**Closest strain based on 16S rRNA gene**	**NCBI accession number**	**% similarity based on 16S rRNA gene**	**Bacteriology**	**Reference**
ST3 C	*Pseudoalteromonas fuliginea* KMM 216	NR_178270.1	97.75	Degrader of complex polysaccharides in marine environments. Not associated with seagrass.	Paulsen et al., 2019
ST3 J	*Vreelandella titanicae* BH1	NR_116997.1	99.56	Marine environments, Sulphur oxidizing	Du et al., 2022

				. Not associated with seagrass.	
SD3G	*Psychromonas arctica* strain Pull 5.3	NR_028821.1	99.66	Psychrophilic marine species. Evidence of associated with seagrass.	Groudieva et al., 2003, Zhang et al., 2024
SD3A	*Variovorax paradoxus* NBRC 15149	NR_113736.1	98.63	Rhizosphere of plants. Produces plant growth promoting hormones but not documented to be associated with seagrass.	Han et al., 2011

To assess the carbohydrate-degrading capabilities of the bacterial isolates, representative genomes were analysed for the presence of carbohydrate-active enzymes (CAZymes) using the dbCAN3 server (dbCAN3, 2024). The HMMR:dbCAN_sub workflow was selected for CAZyme annotation, which leverages hidden Markov models (HMMs) to identify protein domains corresponding to known CAZyme families. The analysis focused on five major classes of CAZymes: glycoside hydrolases (GHs), glycosyltransferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), and auxiliary activities (AAs). Carbohydrate-binding modules (CBMs) were also included to provide insight into substrate specificity and enzyme-target interactions.

The dbCAN_sub pipeline not only identifies CAZyme families but also predicts substrate interactions. This allowed for the functional annotation of each genome's CAZyme repertoire and its likely ecological role in polysaccharide degradation. Substrates such as starch, sucrose, xylan, beta-glucan, chitin, alginate, and peptidoglycan were included in the analysis. Only results that passed the default dbCAN3 significance thresholds (HMMER e-value < 1e-15 and coverage > 0.35) were retained.

To visualize and compare the CAZyme family distribution between the four genomes, a heatmap was produced using RStudio (RStudio Team, 2023) and the ggplot2 package (Wickham, 2016). Stacked bar plots were created to display the number of annotated enzyme subfamilies per genome, grouped by predicted substrate, highlighting functional variation across the four bacterial isolates. These were also RStudio (RStudio Team, 2023) and the ggplot2 package (Wickham, 2016).

Biosynthetic gene clusters (BGCs) were identified using the antiSMASH web server (v7.0.1; Kautsar et al., 2023). The analysis was performed with "relaxed" detection strictness to ensure broader identification of potential clusters. The following settings were enabled: ClusterBlast, SubClusterBlast and KnownClusterBlast, while all other settings were left at their defaults. Genomes analysed included *V. paradoxus*, *V. titanicae*, *P. fuliginea*, and *P. arctica*. Resulting data were processed and visualized in R (RStudio Team, 2023) using the ggplot2 package (Wickham, 2016) to generate pie charts reflecting the distribution and diversity of BGC classes across the different genomes.

3. Results

3.1. Effect of media type on colonies present

This part of the study aimed to determine the number of colonies present on varying growth media of Marine broth (MB/10, Millipore), and F/2 (Sigma-Aldrich) media enriched with ammonium sulphate and sucrose, after an incubation period of 48 hours, at 25°C. The mean CFU for the sediment samples were higher when grown on F2 media, at 51300 ± 294000CFU/g compared to the MB/10 media, at 87000 ± 35300CFU/g (Figure 1). This was not significant a significant difference (t-test results: n=3, df = 3.9277, p-value = 0.2515). The mean CFU for the seed samples were higher when grown on F2 media, at 23700 ± 110000 CFU/seed compared to the MB/10 media, at 33 ± 28.9CFU/g (Figure 1). This result was significant (t-test results: n=3, df = 2.4782, p-value = 0.01308).

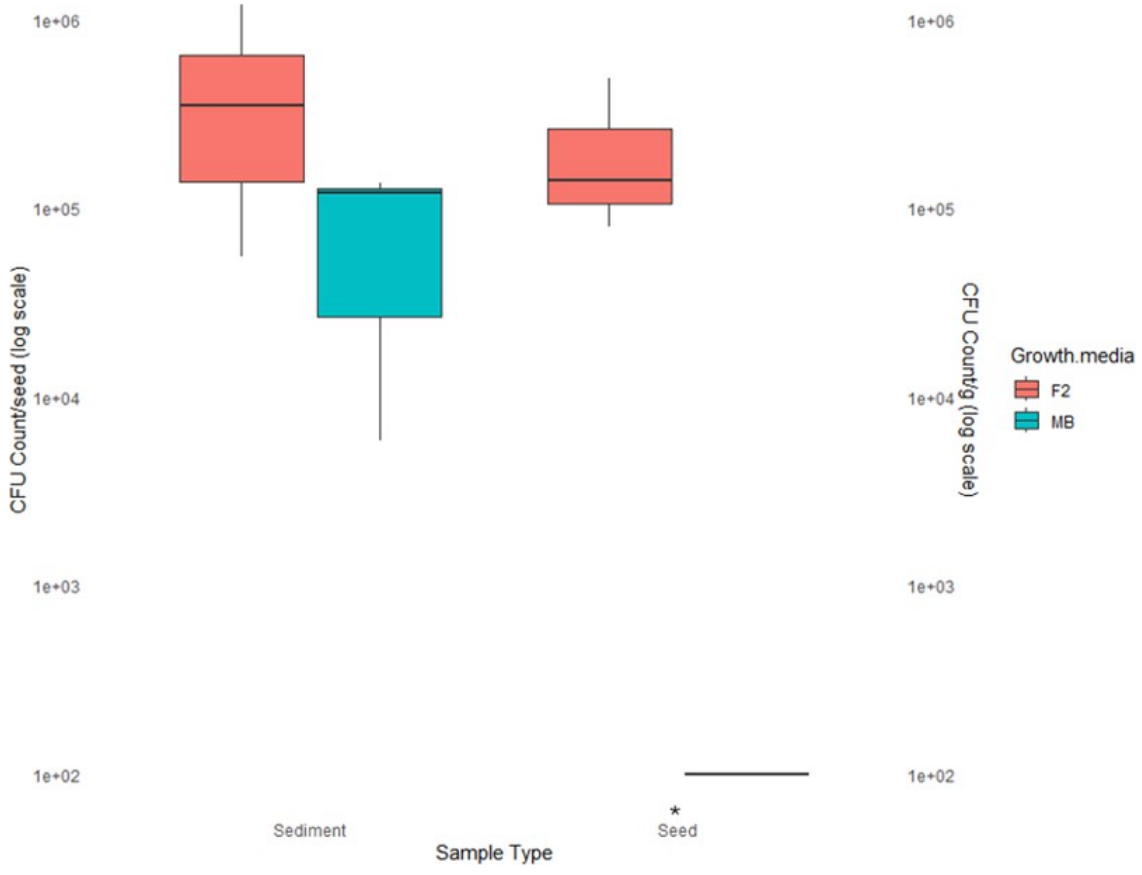

Figure 1 Colony forming units per seed (left axis) and per g of sediment (right axis), grown on either F2 media enriched with ammonium sulphate and sucrose, or MB/10.

3.2. Phylogeny of isolated strains based on 16Ss RNA gene sequences

A total of 20 pure isolates were obtained from both media and sample types. For 18 isolates, amplification and sequencing of the 16S rRNA gene was successful. A phylogenetic tree was constructed from 18 isolated strains and their two closest related type strains obtained from the NCBI database, resulting in an analysis of 36 bacterial strains across nine Orders (Figure 2). Most isolates showed high bootstrap values, such as the *Variovorax* group (with a bootstrap value of 99).

Several relationships, including *Rhodococcus cerastii* with *Rhodococcus yunnanensis* and *Alkalicoccobacillus plakortidis* with *Alkalicoccobacillus murimartini*, had lower bootstrap support (<60), indicating less relatedness with other known strains. Within Alteromonadales, bootstrap values ranged widely (45–100), suggesting substantial variation in relatedness.

Pairwise sequence similarities (Appendix Table 5) revealed that all isolates shared over 97% identity with reference strains, consistent with known species assignments. However, several sequences were near this threshold (e.g., *V. paradoxus* at 98.63% and *Variovorax robiniae* at 97.10%), indicating possible novel species, however the threshold similarity for identification of a new species is <97% (Beye et al., 2017).

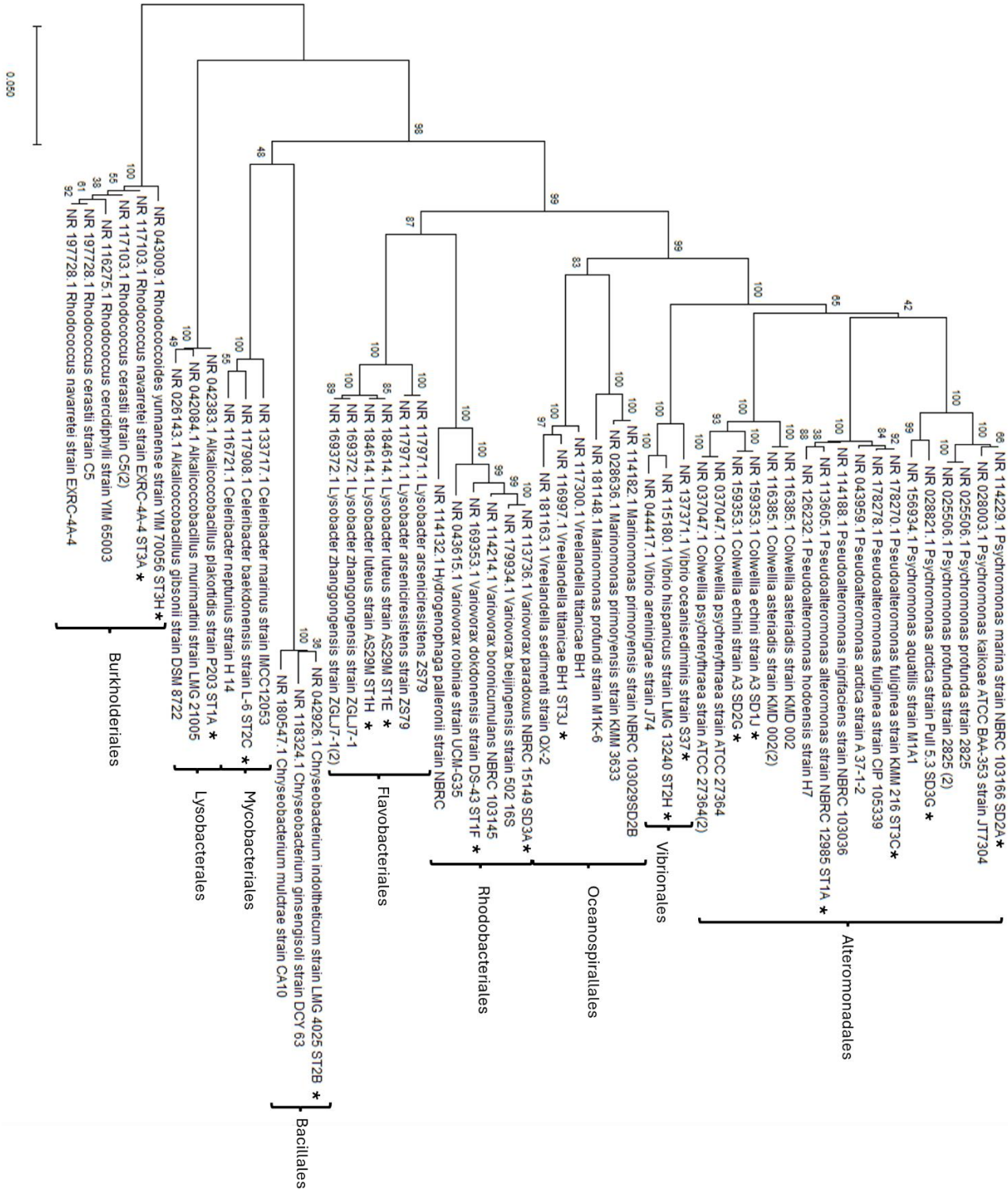

Figure 2 Neighbour-Joining phylogenetic tree (1000 bootstrap replicates) of 16Ss RNA gene sequences sourced from NCBI, including accession numbers and strain ID. The stars represent the strain that is most similar to the bacterial isolate initially sampled from a seed or sediment sample. The tree is rooted with *R. yunnanense*, as it belongs to an Order that is distinct from the four strains selected for full genome sequencing, providing a reference point for evolutionary relationships to be examined. The distance

scale is shown to the bottom left of the phylogram. See Appendix Table 5 for accession numbers.

3.3. Identification of optimal laboratory seagrass germination conditions

To identify optimal germination conditions for seagrass seeds in the laboratory, three different growth media were utilized in preliminary experiments: Agar (1%), sterile sediment, and sterile seawater. The sediment yielded just 8.3% ± 2.5% germination, the sterile seawater yielded 16.7% ± 4.5%, and the highest percentage of germination occurred using agar as the growth media (36.1% ± 8.5%) (Figure 3). Seeds in both the agar and sediment stopped germinating after 15 days; however, the seeds in sterile seawater continued to germinate up until day 21. Results of Fisher's exact test indicated a significant overall difference in germination success across media ($p = 0.044$, $df = 2$, $n = 18$). Post hoc pairwise comparison revealed that germination in agar was significantly higher than in sediment ($p = 0.030$). These findings infer that growth media influences the germination success of seeds, with agar having the largest positive effect.

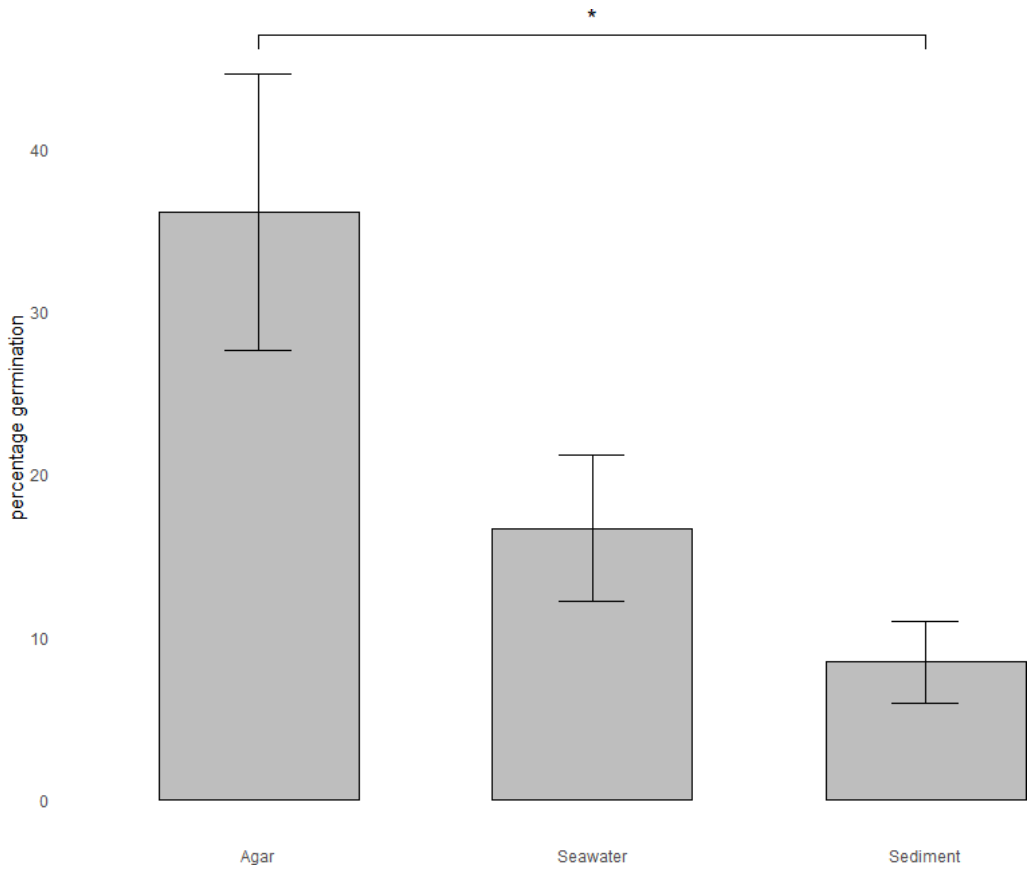

Figure 3. Shows the mean germination percentage (%) of seeds all seeds from grown in three types of growth media and sterile seawater (Agar, sediment, sterile seawater) during the 21-day trial. There were 6 replicates per treatment (n=18). The means for Agar, Seawater, and sediment treatments were 36.1% ± 8.5%, 16.7% ± 4.5%, and 8.3% ± 2.5% respectively.

3.4. Effect of bacterial co-cultivation on cotyledon length

To determine the effect of the isolated strains (appendix, table 7) on germination time, cotyledon length and mortality, seeds were cultivated with bacterial strains for 21 days. After the 21 days, there were no significant differences in cotyledon lengths between each strain after experiment one ($p = 0.6381$, $\chi^2 = 6.08$, $df = 7$, see table 3) or experiment two ($p = 0.4402$, $\chi^2 = 10.00$, $df = 9$, see table 4). *V. paradoxus., C. echini,* and *P. arctica,* mean cotyledon lengths were marginally higher than the control, at 15 mm each ± 7 mm (control without bacteria = 14.3 ± 8.7mm) (Figure 4). *Marinomonas primoryensis*, and *Vibrio hispanicus*., had both the lowest mean cotyledon lengths and smallest standard deviations, at 5.5 ± 2.12 mm and 6.33 ± 2.30 mm (Figure 4).

During the second experiment, numerous co-cultivations, as well as the negative control treatment, exhibited no cotyledon growth at the end of the experiment. Standard deviations about the mean cotyledon length were also very large (± 4mm, *Variovorax robiniae*). As only one measurable cotyledon was produced for *V. titanicae* and *C Echini*, standard deviations were unable to be calculated. The maximum and minimum cotyledon lengths for the combination of the experiments were 32mm and 0mm respectively (Figures 4 and 5).

Table 3, Kruskal Wallace statistical test results for co-cultivation experiment 1, testing for differences between co-cultivation of seagrass seeds with strains 1-8 for each variable (n=32): Days to germinate, Cotyledon length and Mortality.

Experiment 1	p-Value	Chi-squared
Days to germinate	0.253 (df = 7)	10.17
Cotyledon length	0.6381 (df = 7)	6.08
Mortality	0.1157 (df = 7)	12.89

Table 4, Kruskal Wallace statistical test results for co-cultivation experiment 2, testing for differences between co-cultivation of seagrass seeds with strains 9-18 for each variable (n=40): Days to germinate, Cotyledon length and Mortality.

Experiment 2	p-Value	Chi-squared
Days to germinate	0.402 (df = 9)	10.45
Cotyledon length	0.4402 (df = 9)	10.00
Mortality	0.3561 (df = 9)	8.93

3.5. Effect of bacterial co-cultivation on germination time

In the first experiment, the longest mean time to germinate occurred in the co-cultivations with *V. hispanicus*. and *P fuliginea*., at 9.67 ± 3 days, and 8.8 ± 4 days (Figure 4), respectively. The other bacterial isolates caused similar means and deviations, with the control showing the fastest average germination time of 3.1 ± 2.6 days (Figure 4). The difference in germination times between co-cultivations was not significant for either experiment 1 ($p = 0.253$, $\chi^2 = 10.17$, $df = 7$).

The minimum germination occurred in the second experiment, where the control, *Pseudoalteromonas spiralis* and *R. yunnanensis.* co-cultivations, no germination occurred (Figure 5). The fastest germination occurred when co-cultivated with L. luteus, however, this is a non-significant difference as the p-Value was higher than the threshold 0.05 significance ($p = 0.402$, $\chi^2 = 10.45$, $df = 9$).

3.6. Effect of bacterial co-cultivation on seed mortality

In experiments one and two, mortality rates were measured at the end of the trail. In experiment one, at least 50% of seedlings remained alive at the end of the trial period. *Colwellia echini., M. primoryensis, and P. fuliginea*, treatments had the highest mortality (50% of seedlings), compared to *V. paradoxus*, which had a 25% mortality, and higher than the control (at 50%). With a range of just 25% between the lowest and highest mortality, the difference in mortality between the different treatments was not significant ($p = 0.1157$, $\chi^2 = 12.89$, $df = 7$).

Experiment 2 yielded a much higher mortality. Six out of nine tested isolates caused a 100% mortality (Figure 5), however the control group and *V. robinae* treatment yielded zero mortality. This difference is not significant ($p = 0.3561$, $\chi^2 = 8.93$, $df = 9$).

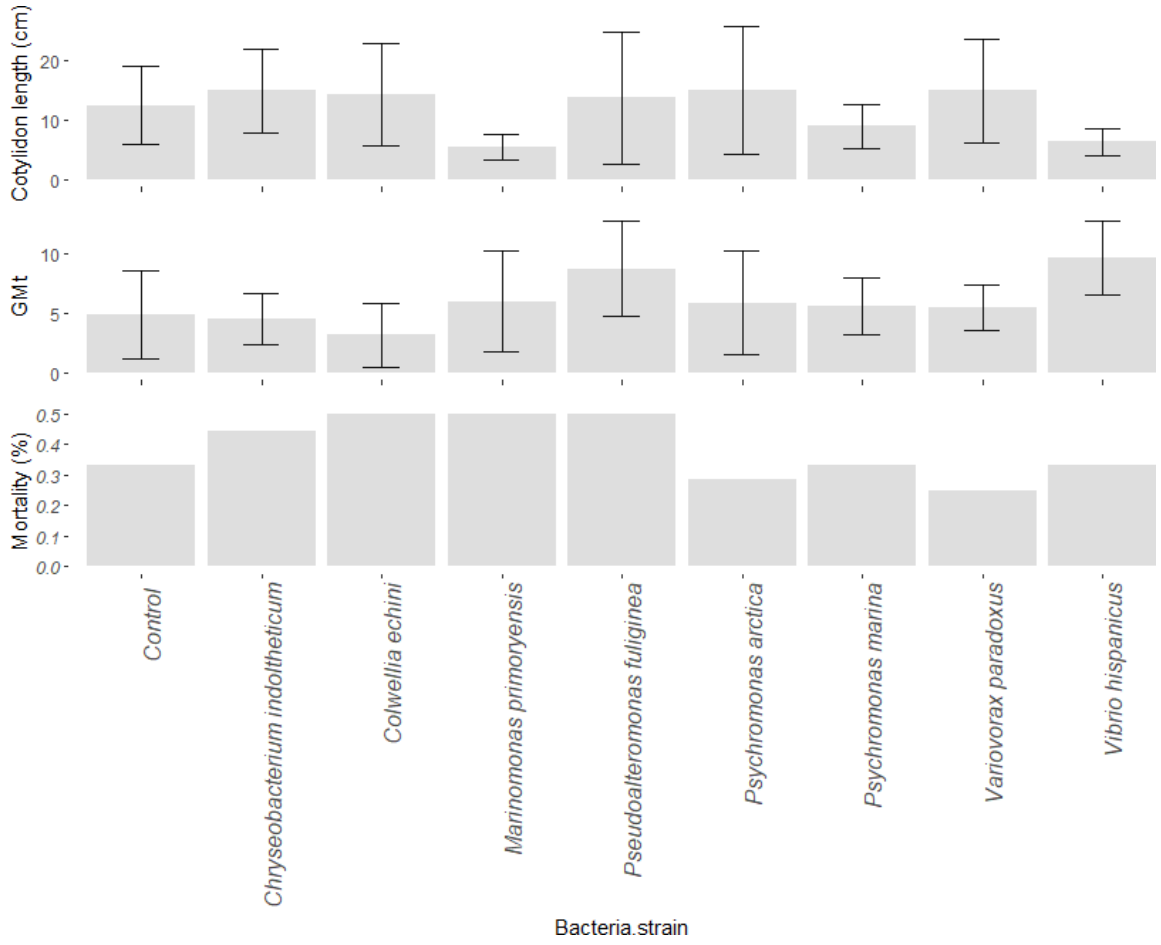

Figure 4, means and standard deviations of seed cotyledon length (top), time to germinate (middle), and mortality (bottom) from co-cultivation experiment 1.

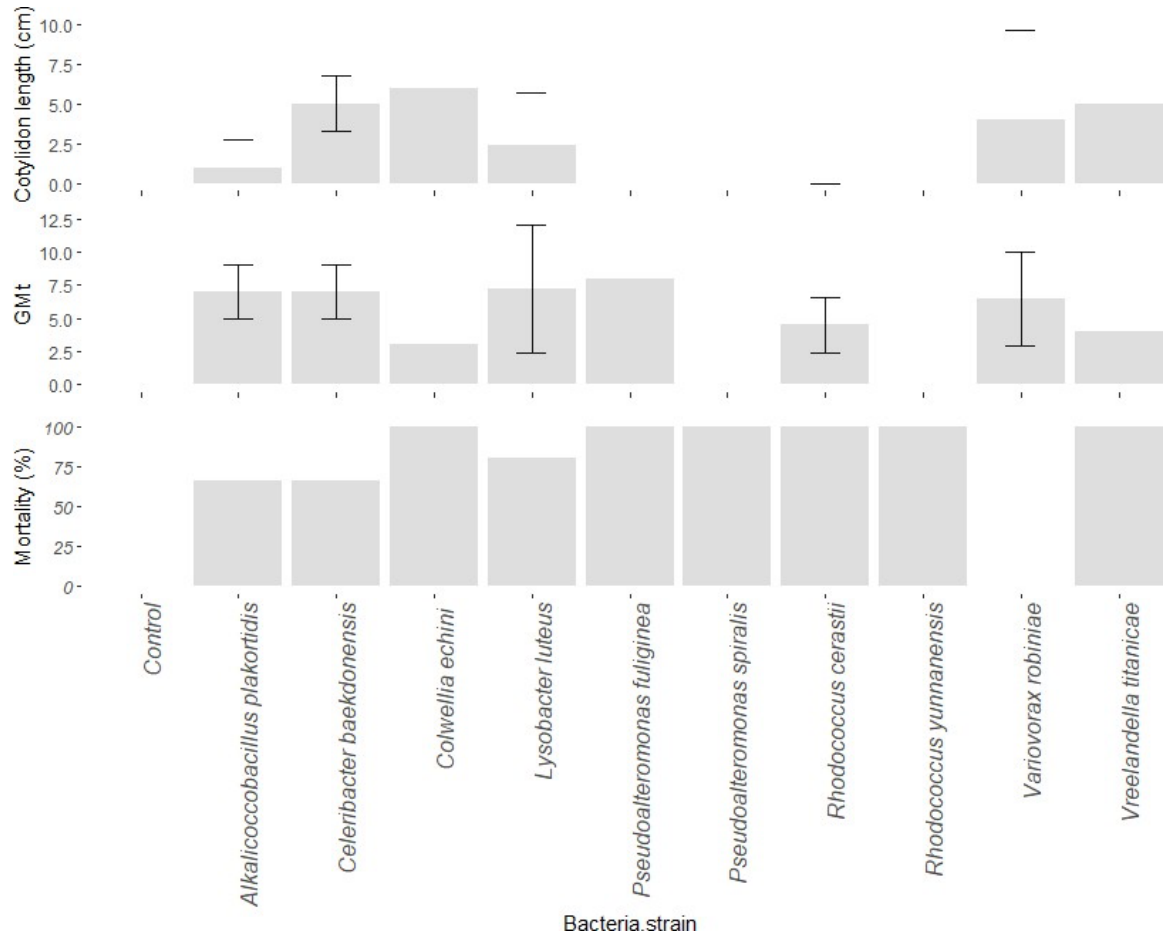

Figure 5, means and standard deviations of seed cotyledon length (top), time to germinate (middle), and mortality (bottom) from co-cultivation experiment 2.

3.7. Analysis of dbcan annotation for CAZymes

The dbcan 3 server was used to determine the presence of CAZymes present in the four genomes that were selected for bioinformatic analysis. *P. fuliginea* is a degrader of marine polysaccharides (Paulsen et al., 2019), *V. titanicae* as it reduces harmful compounds such as the oxidation of sulphur (Du et al., 2022). *P. arctica* is a psychrophilic bacterium, associated with seagrass (Groudieva et al., 2003; Zhang et al., 2024). Finally, *V. paradoxus* was selected as some strains are known to produce growth promoting hormones, increasing growth in agricultural plants (Han et al., 2011).

P. fuliginea exhibited the largest number of CAZymes, and the greatest variation, as shown in figure 6. The PL and CE families were only present in this genome, which is a further indication of the variation of CAZymes within the genome. The other genomes exhibited a similar presence of CAZyme's, with CB, GH, and GT being the dominant ones across the four. The AA family was the least represented across the four species, with a max of eight (*V. titanicae*) subfamilies and minimum of zero (*P. arctica*) (Figure 6).

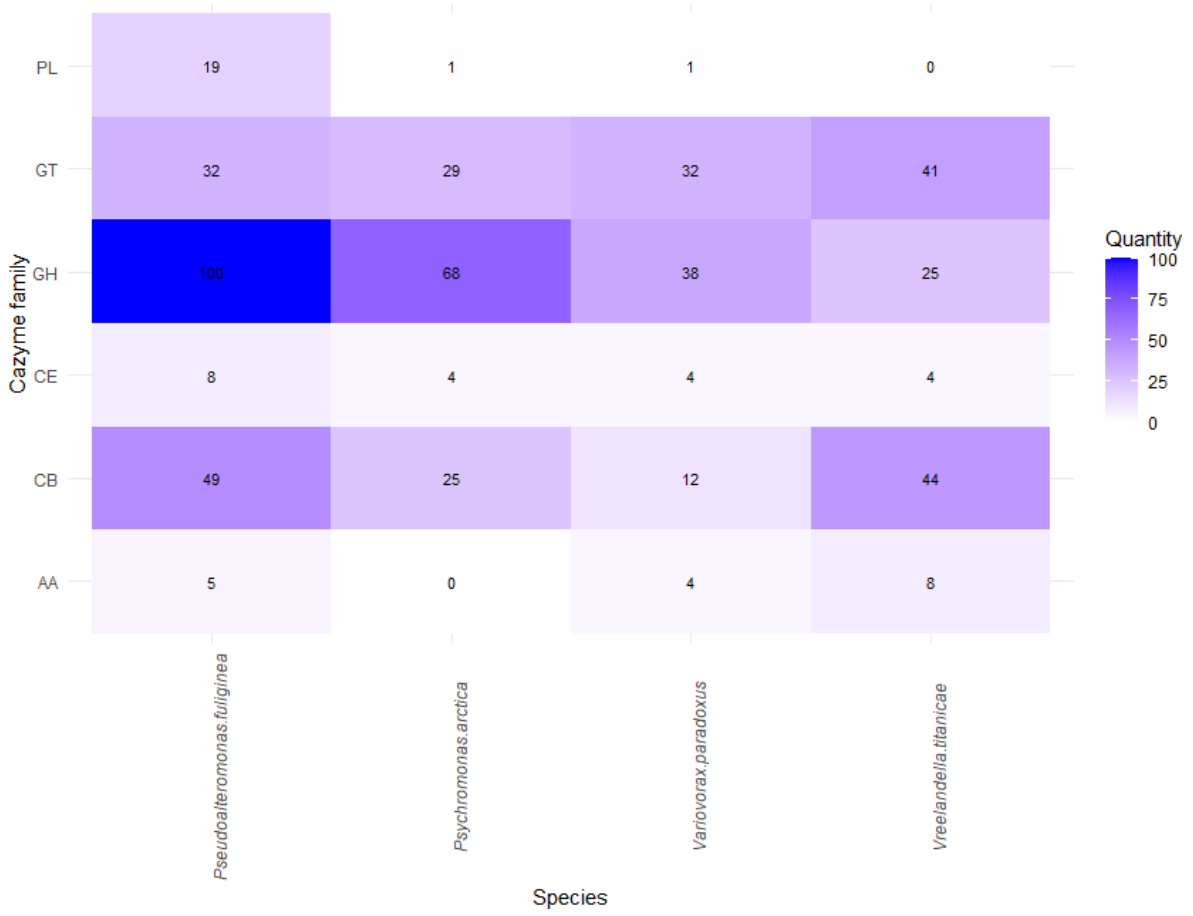

Figure 6. Heatmap showing the abundance and diversity of CAZyme families—auxiliary activities (AA), carbohydrate esterases (CE), glycoside hydrolases (GH), glycosyltransferases (GT), and polysaccharide lyases (PL)—across four genomes analysed using the dbCAN3 server (dbCAN3, 2024).

Stacked bagplots were created to show the variation and number of subfamilies encoded within each genome annotated. *V. paradoxus* **(Figure 7)**, was predicted to contain the least CAZyme subfamilies (91), 52 were without substrate predictions. There were nine predicted substrates to the remaining 39 CAZyme families. Starch and sucrose were predicted substrates to the most CAZymes, with 10 and 11 CAZyme subfamilies, respectively.

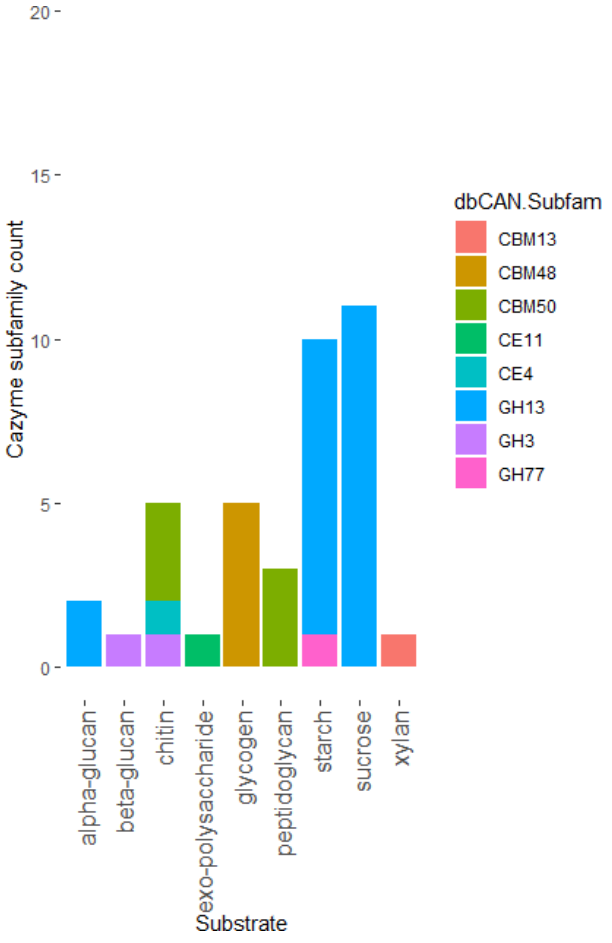

Figure 7, Annotation of Carbohydrate active enzymes families in *V. paradoxus* and the predicted substrates that the respective CAZymes interact with.

P. fuliginea **(Figure 8)** had both the largest number of CAZyme families out of the four genomes anotated, and the highest number of predicted substrates. 70 CAZyme's had no predicted substrate and the remaining 143 interacted with 22 different substrates. Contrary to *V. paradoxus* and *P. arctica*., substrates beta glucan and Xylan had the most subfamilies, with 20 each. Alginate, chitin and starch were also substrates to >13 CAZymes.

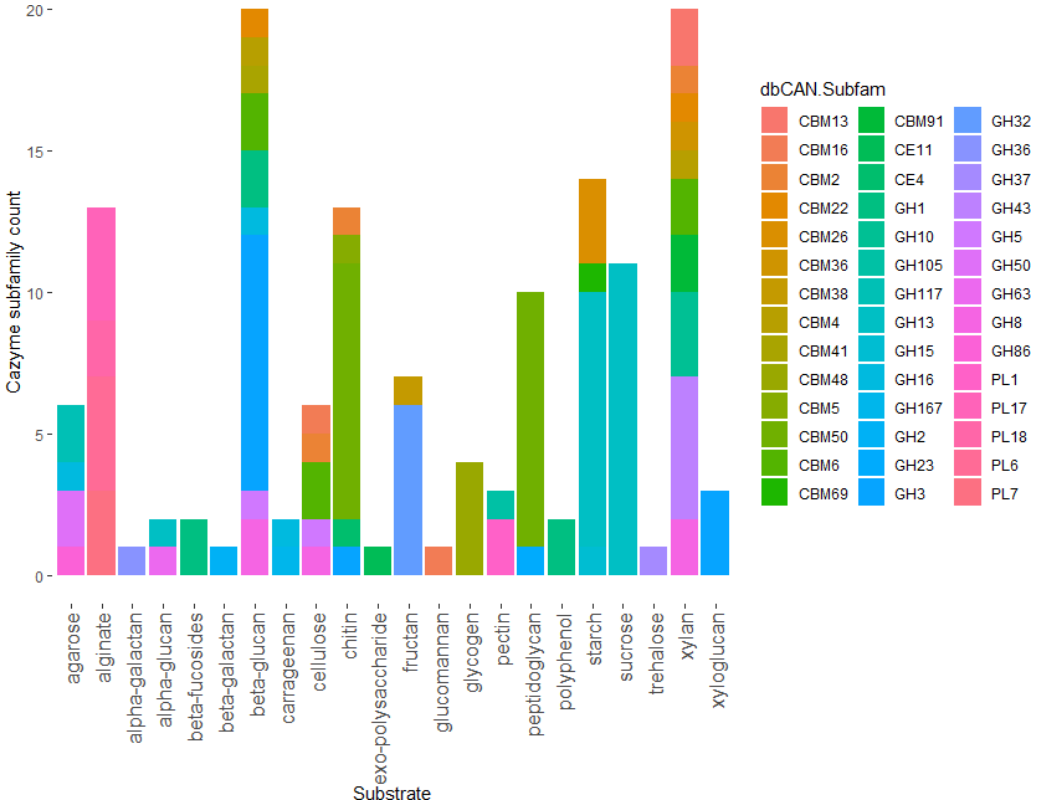

Figure 8, Annotation of Carbohydrate active enzymes families in *P. fuliginea* and the predicted substrates that the respective CAZymes interact with.

P. arctica **(Figure 9)** had a similar distribution of CAZymes and substrates to *V. paradoxus*, however with nearly double the subfamilies with sucrose and starch substrates at 20 and 14, respectively. Beta glucan had 12 interacting CAZymes and chitin had 7. Additionally, there were 6 subfamilies with polypeptoglycan substrates.

There were 127 total subfamilies for *P. arctica*, 48 of which did not have a predicted substrate.

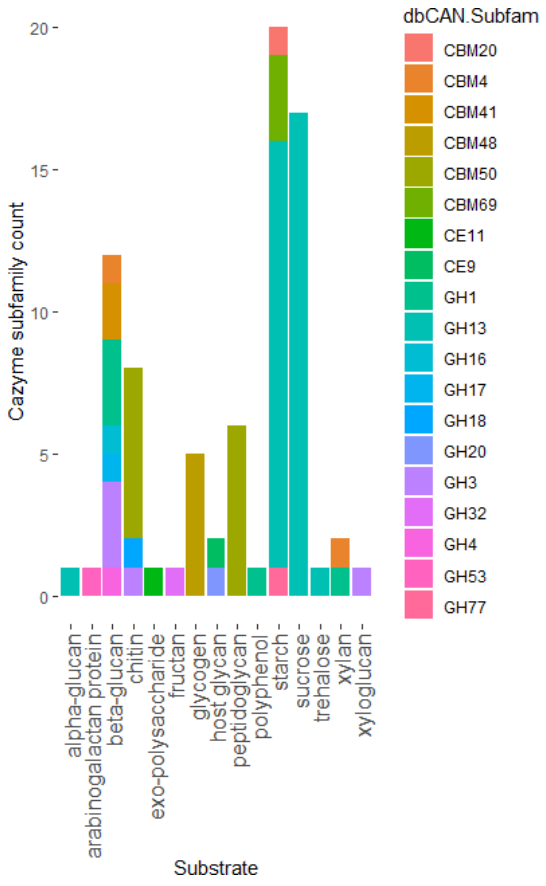

Figure 9, Annotation of Carbohydrate active enzymes families in *P. arctica* and the predicted substrates that the respective CAZymes interact with.

V. titanicae **(Figure 10)**, had the least subfamilies with predicted substrates of starch and sucrose (2 and 3 respectively). There were 15 subfamilies with xylan predicted substrates: the second highest of the four bacteria genomes. There were 122 subfamilies of CAZyme's for *V. titanicae*, however more than half of these were without a predicted substrate.

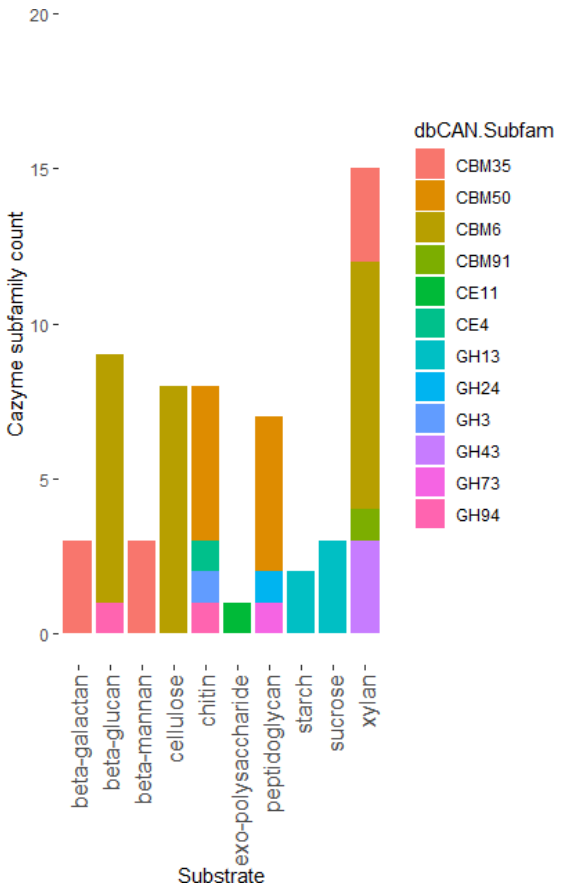

Figure 10, Annotation of Carbohydrate active enzymes families in *V. titanicae* and the predicted substrates that the respective CAZymes interact with.

3.8. Bioinformatic analysis of biosynthetic gene clusters

The antismash pipeline (v7.0.1; Kautsar et al., 2023) was used to determine the biosynthetic gene clusters present in the four genomes *V. paradoxus*, *V. titanicae*, *P. fuliginea*, and *P. arctica. V. paradoxus* exhibited the highest diversity, with clusters including NRPS, PKS, RiPP-like, arylpolyene, and hybrid types (e.g., T1PKS-NRPS) totalling 11 different clusters. In contrast, *V. titanicae* contained only three gene clusters, consisting primarily of RiPP-like clusters. Both *P. fuliginea* (three different clusters) and *P. arctica* (six different clusters) shared

the presence of hybrid NRPS clusters but differed in other cluster types such as ectoine, betalactone, and butyrolactone. The variation in gene clusters present in each genome is evident in figure 11.

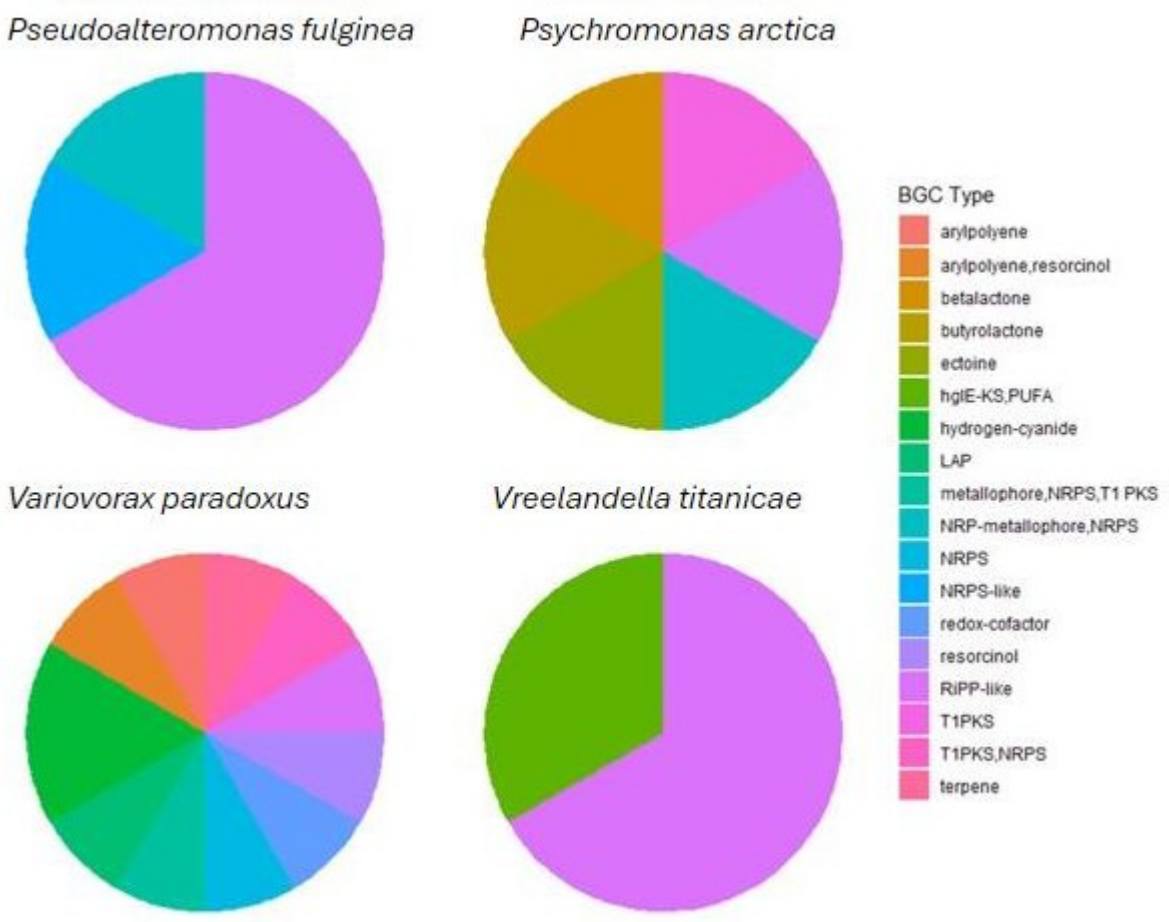

Figure 11. Composition of biosynthetic gene clusters (BGCs) in four bacterial genomes. Pie charts show the relative distribution of BGC types identified by antiSMASH in *V. paradoxus*, *V. titanicae*, *P. fuliginea*, and *P. arctica*. Cluster classes include NRPS, PKS, RiPP-like, and various specialized or hybrid types. Segment sizes reflect the number of clusters per type within each genome.

4. Discussion

4.1. Overview

By studying seagrass associated bacteria, we can further understand the relationship that the microbiome has with seagrass, and aid restoration efforts of these diverse and ecologically

important ecosystems. We investigated the influence of nutrients in media on bacterial growth, and the effects of media types on gemmation, to determine the optimum growth conditions in a lab setting.

The co-cultivation of seagrass associated bacteria and seagrass seeds is a pioneer study, investigating whether individual species affect germination success. This study also increases the knowledge on seagrass associated bacteria and the functional relationships between them, through the utilization of bioinformatics. By isolating bacteria and sequencing their 16s RNA genes and the full genomes of four selected strains, we were able to identify some of the functional roles that the bacteria play in nutrient cycling and potential plant growth promotion. The following discussion evaluates these findings in the context of ecological function, microbial biotechnology, and their implications for future seagrass restoration strategies.

4.2. Comparison of bacterial growth medium

Bacteria associated with different parts of the seagrass microbiome require different nutrients to grow and function (Prasad et al., 2018). As a result, microbes isolated from the seed surface produced almost no colonies on the marine broth media, compared to the F/2 solution media (supplemented with Ammonium sulphate and sucrose). Similar findings occurred in a study by De Rosa et al., 2003, who found that microbes grow better on growth media with readily available carbon sources, such as glucose, as opposed to marine broth media. Furthermore, bacteria produce 3 times more metabolites when grown on media with easily accessible sources of carbon, promoting further bacterial growth (Deniz Tasdemir et al., 2023).

4.3. 16S gene sequences: determining functional roles

16S rRNA sequencing was used to investigate the taxonomic classification of the bacterial isolates. This section discusses the major bacterial orders identified in the study through the sequencing of the 16S RNA gene, with a focus on their ecological functions and relevance to the seagrass microbiome.

Members of the Order Alteromonadales, were highly abundant in this study, encompass a wide range of species, including strains such as *C. echini*, commonly found in sea urchins (Christiansen et al., 2018), and psychrotolerant species like *Pseudoalteromonas arctica* and *Psychromonas arctica* (Al Khudary et al., 2010; Corsaro et al., 2008). Species such as these also play important roles in nutrient cycling (Liu et al., 2018).

An imbalance in nutrient availability may create favourable conditions for the proliferation of pathogenic species (Daniel et al., 2017). For instance, bacteria within the order Oceanosprillales have been found in areas of oil pollution. They have potential to mitigate the effects of hydrocarbon pollution by degrading aliphatic hydrocarbons (Diogo Jurelevicius et al., 2013). Seagrass isolates from the orders Burkholderiales (Jiang et al., 2015), Mycobacteriales, Rhodoccocus can also degrade these pollutants, thus promoting ecosystem health and seagrass growth (Jamal and Pugazhendi, 2020).

4.4. Preliminary experiment: growth media

The preliminary experiment investigated the effect of growth media on successful germination. Agar yielded significantly higher germination (36.1% ± 8.5%) than the other media types, of sediment and sterile seawater. Li et al., 2021 found similar results, with seeds grown in agar yielding higher germination than those grown in sediment. A possible explanation for this is that agar provides favourable growth conditions, with soft substrate for the root to attach too, and providing anaerobic conditions that have been shown to produce higher germination than aerobic conditions (Probert and Brenchley, 1999).

The 36.1% ± 8.5% germination success found in the agar growth medium treatment is higher than that which typically occurs in nature, where seedling emergence can be as low as 5% (Unsworth et al, 2024). This has implications in assisting restoration efforts, where planting techniques are still being researched to be more effective (Balestri and Lardicci, 2012). Despite seedlings growing faster in agar in a lab setting, further studies should investigate the plantation technique in the wild. Especially as agar begins to degrade after three weeks, making it difficult for roots to establish a stable matrix (Li et al., 2021). Successfully germinated seeds can however be transplanted into sediments as research by Orth et al., 2009, whose investigations on seedling transplantation from agar yielded successful results.

4.5. Co-cultivation experiments

The first co-cultivation trial indicated that some strains, e.g. *V. paradoxus* and *P. arctica,* may promote growth of seedlings, as suggested by longer cotyledons. While studies investigating the effect of bacteria on seagrass germination and growth have not been well investigated, there have been similar investigations on agricultural crops, such as wheat. Gong et al., 2023 found that bacterial seed coatings of *Pontibacter actiniarum,* significantly increased germination

success and growth rate of the crop. This may have implications for marine plants, confirming that marine bacteria can positively affect the growth and germination of plants.

The second co-cultivation trial yielded poor results compared to the first. Despite the higher number of strains investigated, fewer seeds successfully germinated, and had a higher mortality rate, up to 100%. These experiments occurred a month after the first experiment was completed.

4.6. Implications for seed storage

While Marion and Orth, 2008., found that *Zostera marina* maintained high viability (92–95%) over a 3-month storage period, seeds held in storage are more susceptible to parasites and disease (Dooley et al., 2013), compromising the chance of successful germination. It is then important to implement techniques to separate viable, healthy seeds from non-viable seeds to increase the efficiency of restoration efforts, using stored seeds (Marion and Orth, 2008)

There is evidence to suggest treating seeds with copper sulphate and nano-silver, providing antimicrobial resistance, and increasing the health of seedlings (Sullivan et al., 2022; Xu et al., 2019), may increase the time seeds can be stored for, without reducing their chances of germination. This is particularly relevant given the outcomes of the co-cultivation experiments: Experiment 1, which used fresher seeds, showed significantly higher germination and seedling survival compared to Experiment 2, where seeds stored for a longer period experienced up to 100% mortality. These results emphasize the time sensitivity of seed storage and highlight the importance of integrating antimicrobial treatments and viability screening to improve restoration scalability and success.

4.7. Bioinformatic analysis

Bioinformatic techniques were used to investigate functional roles of the four genomes of *P. fuliginea, V. titanicae, P. arctica* and *V. paradoxus* Each strain annotated by the dbCAN server produced enzymes that break down sugars produced by seagrass. Using the dbCAN server, the genomes of the four strains (what were they) were annotated to identify carbohydrate-active enzymes (CAZymes) that could degrade these components. This approach allows a detailed understanding of the metabolic potential and ecological roles of seagrass-associated bacteria (Zheng et al., 2022).

To further investigate the bioactive functions of these bacteria, the antiSMASH database was used to annotate biosynthetic gene clusters. This provided insight into the potential secondary metabolites produced by the species, which may influence their interactions with seagrass. For example, *P. fuliginea* was found to produce five unspecified ribosomally synthesized and post-translationally modified peptides, which have highly diverse functions including antimicrobial activities and signalling (Zhong et al., 2020). While low in similarity, antimicrobial gene clusters were additionally identified to produce indigoidine and delfibactins A and B, both known for their antifouling properties. (Xu et al., 2015; Tejman-Yarden et al., 2019). Antifouling is described as reducing the number of inhibitory or pathogenic bacteria around the host species (Satheesh et al., 2016). However, the cocultivation of *P. fuliginea* yielded a 100% mortality at the end of the trial, which might be linked to its production of degradative enzymes that break down polysaccharides, including cellulose in the cell wall (Hobbs & Boraston, 2024). While these degradative activities could damage the seagrass seedlings, *P. fuliginea* may still be beneficial by enhancing nutrient cycling and facilitating seagrass growth through degradation of dead plant matter (Liu et al., 2018). *P. fuliginea* is also unique to the other genomes, in that it is the only genome with alginate and agarose substrates. Both Carbohydrates make up algal matter. The breakdown of algal matter may have both positive and negative impacts on seagrass growth, due to the complex nature of the relationship between algae and seagrasses. Algae can either promote or inhibit the growth and health of seagrass (Leemans et al., 2020; Wear et al., 1999).

P. arctica, is a psychrophilic strain typically found in arctic seawater (Corsaro et al., 2008). This species is well adapted to cold environments due to modifications in its outer membrane, including altered lipopolysaccharide structures, allowing it to thrive in temperatures between 0°C and 10°C (Groudieva et al., 2003). Additionally, antiSMASH revealed that *P. arctica* produces ectoine. Ectoine stabilizes nucleic acids and proteins in extreme environments, providing further protection under high salinity and cold conditions (Gao et al., 2013). Antibacterial metabolites plipastatin and butyrolactone (Abdel-Mageed et al., 2020; Satheesh et al., 2016) were also annotated, which reduce the effect of pathogens, and may explain the low mortality rates observed in growth trials.

V. titanicae (Basionym *Halomonas*) had the highest number of GT family CAZymes, inferring that the species provides functions other than nutrient cycling. Literature supports the role of *Halomonas* species in promoting cell growth by production siderophore's, phosphate reduction

(Aires et al., 2021), and sulphur oxidation (Du et al., 2022). It is also a nitrogen fixing species, increasing the availability of nitrogen for uptake in seagrass (Lu et al., 2024). Furthermore, *V. titanicae* possesses halophilic properties that help reduce stress in high-salinity environments, potentially benefiting seagrasses in symbiotic relationships (Aires et al., 2021).

Eicosapentaenoic acid was predicted by antiSMASH to be produced by *V. titanicae*. This fatty acid is found in seagrasses in low concentrations (Beca-Carretero et al., 2020). Seagrasses can absorb whole carbon-based molecules such as polyunsaturated acids directly from the environment, unlike nitrogen that must be reduced and cycled for uptake (Van Engeland et al., 2013). As a result, fatty acids produced by bacteria such as *Halomas sp*. Can be utilized by seagrass. These fatty acids are crucial for plant nutrition, growth, and reproduction (Parrish, 2009). Omega-3 fatty acids are particularly useful for seagrasses in colder environments, contributing to thermoregulation and serving as an energy source (Beca-Carretero et al., 2020).

The bioinformatic analysis revealed the presence of diverse biosynthetic gene clusters, and carbohydrate-active enzymes across just four genomes, highlighting the functional and ecological versatility of seagrass-associated bacteria. CAZymes enable the degradation of structurally complex components of the seagrass cell wall, including lignocellulose, xylans, and storage carbohydrates, suggesting roles in nutrient cycling. Secondary metabolite predictions further revealed that these bacteria produce compounds with antimicrobial, signalling, and stress-protective properties. *P. fuliginea* synthesizes antifouling compounds but may also contribute to seedling mortality via overactive degradative pathways. In contrast, *P. arctica* exhibits traits linked to cold and salt tolerance, while *V. titanicae* stands out for its potential to supply beneficial fatty acids and promote nutrient availability through siderophore production and nitrogen fixation. The diverse functional roles that the bioinformatic analysis of just four genomes highlights the importance of the whole microbiome to seagrasses and reveals the complexity of the relationship between the two. Understanding these relationships is critical for predicting ecosystem responses to environmental change and for informing conservation or restoration strategies involving plant-microbe interactions.

4.8. *V. paradoxus*

Although *V. paradoxus* showed no significant effect in the germination experiment, previous studies suggest it could have a positive effect on the growth and health of seagrass while potentially benefiting from plant-derived nutrients. Starch found in rhizomes may be broken

down for energy, and sucrose has been shown to be secreted into the sediment by seagrasses, which might be made available for beneficial bacteria to consume (Sogin et al., 2022; Burke et al., 1996). In turn, *V. paradoxus* is thought to produce secondary metabolites that may support the growth and health of the plant. The secondary metabolite arylopeylene, annotated by antiSMASH, has been associated with antioxidant functions (Schöner et al., 2016), which could help mitigate the effects of toxins and support plant health (Schmalenberger et al., 2008).

V. paradoxus has been identified as a candidate for potential agrobiotechnological applications, including use as a biofertilizer (Han et al., 2011), since some strains have been found to produce indole-3-acetic acid, a plant growth-promoting hormone (Tarquinio et al., 2019). In agriculture, biofertilizers derived from seagrass isolates have been reported to increase crop growth (Saranya et al., 2022) and may serve as a less environmentally damaging alternative to conventional fertilizers (Yu et al., 2024). However, there is a research gap regarding the use of biofertilizers on seagrass. Therefore, future studies should consider investigating their effects on seagrass germination as a potentially natural method for promoting growth in restoration contexts.

5. Conclusion

The preliminary experiment demonstrated that agar medium enhances germination success for seagrass seeds, yielding a 36.1% germination rate compared to lower rates typically observed in natural settings (Unsworth et al., 2024). Agar medium showed promise for enhancing germination under laboratory conditions, before being transplanted into a natural environment. Further investigations should determine the response of a seed growing in a controlled environment, then being transplanted into a natural environment for restoration purposes.

The co-cultivation experiments showed results that were not significant. However, isolates such as *V. paradoxus* and *P. arctica* may increase germination success, as the mean cotyledon lengths at the end of the trial were longer than the control. Bacteria such as these, that comprise the seagrass microbiome play significant and functionally diverse roles, contributing to nutrient cycling, antifouling, and plant growth (Graham et al., 2024). Through the production of carbohydrate-active enzymes (CAZymes) and secondary metabolites, these bacteria facilitate nutrient exchange and protection, potentially fostering mutualistic relationships with seagrass. There is growing interest in the potential use of beneficial bacteria in restoration efforts, such as using seagrass-associated bacteria like *V. paradoxus* as biofertilizers (Saranya et al., 2022),

providing a natural alternative to synthetic fertilizers that often have detrimental effects on surrounding ecosystems. Given the ongoing challenges posed by pollution and climate change to seagrass habitats (Ren et al., 2024), further research should be undertaken to investigate the long-term effects of biofertilizers on seagrass health and resilience, specifically examining their impacts on germination rates, nutrient uptake, and overall ecosystem functioning.

6. Appendix

Appendix Table 5, Summary of strains isolate, and the media they were initially grown on

Strain Number	**Closest type strain**	**Similarity**	**Source**	**Strain ID**	**Growth Media**	**NCBI accession**
1	*Chryseobacterium indoltheticum strain LMG 4025*	99.91%	Seed	St2B	MB/10	NR_042926.1
2	*Psychromonas arctica strain Pull 5.3*	99.66%	Seed	Sd3G	F2 + Ammonium sulfate +Sucrose	NR_028821.1
3	*Vibrio hispanicus strain LMG 13240*	97.13%	Seed	St2H	F2 + Ammonium sulphate +Sucrose	NR_115180
4	*Colwellia echini strain A3*	97.13%	Seed	Sd1J	F2 + Ammonium sulphate +Sucrose	NR_159353.1

5	*Psychromonas marina strain NBRC 103166*	97.74%	Seed	Sd2A	F2 + Ammonium sulphate +Sucrose	NR_114229.1
6	*Marinomonas primoryensis strain NBRC 103029*	98.90%	Sediment	Sd2B	F2 + Ammonium sulphate +Sucrose	NR_114182.1
7	*Pseudoalteromonas fuliginea strain KMM 216*	99.75%	Sediment	St3C	F2 + Ammonium sulphate +Sucrose	NR_178270.1
8	*Variovorax paradoxus NBRC 15149*	98.63%	Seed	Sd3A	F2 + Ammonium sulphate +Sucrose	NR_159353.1
9	*Colwellia echini strain A3*	97.77%	Seed	Sd2G	F2 + Ammonium sulphate +Sucrose	NR_113736.1
10	*Pseudoalteromonas spiralis strain Te-2-2*	99.47%	Sediment	St1A	F2 + Ammonium sulphate +Sucrose	NR_114801.1
11	*Lysobacter luteus strain AS29M*	99.83%	Sediment	St1E	F2 + Ammonium sulphate +Sucrose	NR_184614.1

12	*Variovorax robiniae strain UCM-G35*	97.10%	Sediment	St1F	MB/10	NR_169353.1
13	*Lysobacter luteus strain AS29M*	99.83%	Sediment	St1H	MB/10	NR_184614.1
14	*Alkalicoccobacillus plakortidis strain P203*	99.83%	Sediment	St2A	MB/10	NR_042383.1
15	*Celeribacter baekdonensis strain L-6*	99.70%	Sediment	St2C	MB/10	NR_117908.1
16	*Rhodococcus cerastii strain C5 16S*	99.13%	Sediment	St3A	F2 + Ammonium sulphate +Sucrose	NR_117103.1
17	*Rhodococcus yunnanensis STRAIN*	98.71%	Sediment	St3H	F2 + Ammonium sulphate +Sucrose	NR_043009.1
18	*Vreelandella titanicae STRAIN*	99.56%	Sediment	St3J	F2 + Ammonium sulphate +Sucrose	NR_116997.1

Appendix Table 6, secondary metabolites produced by the antiSMASH server (K et al., 2023) used to investigate biosynthetic gene clusters (version 7.0.1) encoded in *Variovorax paradoxus*, with a relaxed strictness and the default settings

Region	Type	From	To	Most similar known cluster	Similarity	%
1.1	arylpolyene	288,994	330,196			
1.2	hydrogen-cyanide	345,840	358,687			
1.3	LAP	2,232,957	2,255,143			
1.4	resorcinol	2,759,199	2,801,142			
1.5	terpene	3,342,766	3,364,513			
1.6	redox-cofactor	3,412,571	3,434,776			
1.7	T1PKS,NRPS	3,538,071	3,590,423	colanic acid	Saccharide	9
1.8	arylpolyene,resorcinol	3,753,892	3,800,203	lipopolysaccharide	Saccharide:Lipopolysaccharide	5
1.9	NRP-metallophore,NRPS,T1PKS	4,128,340	4,204,204	variochelin A/variochelin B	NRP+Polyketide	65
1.10	NRPS	4,341,278	4,388,774	bacillibactin	NRP	28
2.1	RiPP-like	309,288	320,130			
2.2	hydrogen-cyanide	737,232	750,186			

Appendix Table 7, secondary metabolites produced by the antiSMASH server (K et al., 2023) used to investigate biosynthetic gene clusters (version 7.0.1) encoded in *P. fuliginea*, with a relaxed strictness and the default settings

Region	Type	From	To	Most similar known cluster	Similarity	%
9.1	RiPP-like	29,113	39,970			
10.1	NRP-metallophore,NRPS	14,773	63,888	delftibactin A/delftibactin B	NRP+Polyketide	10
13.1	RiPP-like	58,700	69,605			
19.1	RiPP-like	36,889	48,649			
24.1	NRPS-like	22,389	66,267	indigoidine	Saccharide	20
26.1	RiPP-like	146,558	156,420			

Appendix Table 8, secondary metabolites produced by the antiSMASH server (K et al., 2023) used to investigate biosynthetic gene clusters (version 7.0.1) encoded in *Psychromonas arctica*, with a relaxed strictness and the default settings

Region	Type	From	To	Most similar known cluster	Similarity	
Region 1	RiPP-like	2,371,324	2,383,507			
Region 2	ectoine	3,644,988	3,655,380	ectoine	Other	75%
Region 3	betalactone	4,020,361	4,043,056	plipastatin	NRP	15%
Region 4	T1PKS	4,380,985	4,428,397			

Region 5	NRP-metallophore,NRPS	5,055,085	5,119,170	potashchelin A/potashchelin B/potashchelin C/potashchelin D	NRP	78%

Region 6	butyrolactone	5,251,238	5,262,167			

Appendix Table 9, secondary metabolites produced by the antiSMASH server (K et al., 2023) used to investigate biosynthetic gene clusters (version 7.0.1) encoded in *V. titanicae*, with a relaxed strictness and the default settings.

Region	Type	From	To	Most similar known cluster	Similarity	
Region 1.1	hglE-KS,PUFA	342,091	399,240	eicosapentaenoic acid-like compound	Other	13%
Region 21.1	RiPP-like	1	5,908			
Region 50.1	RiPP-like	8,735	14,417		Vreelandella	

7. RStudio code

```
Media.type <- read.csv("C:/Users/Gilbert Jones/Downloads/Media.type.csv",
stringsAsFactors=TRUE)

Media.type <- Growth.media.boxplot

install.packages("ggplot2")
library(ggplot2)
p1 <- (ggplot(Media.type, aes(x=Growth.media, y=Percentage.germination,
group=Growth.media, color = Growth.media)) +
       geom_bar() +
       labs(x = "Time (Days)", y = "Percentage germination") +
     guides(fill = guide_legend(title = "Growth media")) +
     theme_classic())
p1
```

```
ggplot(Cumulative.germination, aes(x = Growth.media, y = Percentage.germination, fill =
Growth.media)) +
  geom_bar(stat = "identity", width = 0.6, color = "black") +  # Bars with black border
geom_errorbar(aes(ymin = Percentage.germination - sd, ymax = Percentage.germination +
sd), width = 0.2) +  # Error bars
  labs(title = "Barplot with Error Bars", x = "Category", y = "Value") +
  theme_minimal()


Cumulative.germination <- data.frame(
                    Growth.media = c("Seawater", "Agar", "Sediment"),
                    Percentage.germination = c(16.7, 36.1, 8.5),
                    sd = c(4.5, 8.5, 2.5))

ggplot(Cumulative.germination, aes(x = Growth.media, y = Percentage.germination, fill =
Growth.media)) +
  geom_bar(stat = "identity", width = 0.6, color = "black", fill = "grey") +  # Bars with black
border
  geom_errorbar(aes(ymin = Percentage.germination - sd, ymax = Percentage.germination +
sd), width = 0.2) +  # Error bars
  theme_minimal()+
  theme(panel.grid = element_blank(),  # Remove gridlines
        panel.background = element_blank())+
  labs(x = "", y = "percentage germination")+ geom_signif(comparisons = list(c("Agar",
"Sediment")),
                                        annotations = "*",
                                        y_position = 45,  # Adjust height above bars
                                        tip_length = 0.02,
                                        textsize = 5)
install.packages("ggsignif")
library(ggsignif)
install.packages("ggplot2")
library(ggplot2)

p1 <- (ggplot(Media.type, aes(x=Germination.time..days,
y=Germination.cumulative.frequency..., group=Growth.media, color = Growth.media)) +
        geom_point() +
        geom_line(color="grey", alpha=0.5) +
        labs(x = "Time (Days)", y = "Percentage germination") +
        guides(fill = guide_legend(title = "Growth media")) +
        theme_classic())
p1
Plot.GMT1.1 <- ggplot(my_sum1.1) + geom_bar( aes(x=Bacteria.strain, y=mean),
stat="identity", fill="grey", alpha=0.5) + geom_errorbar( aes(x=Bacteria.strain, ymin=mean-
sd, ymax=mean+sd), width=0.3, colour="black", alpha=0.9, size=0.5) + theme(axis.text.x =
element_blank(), axis.title.x = element_blank()) + labs(y = "GMt") + theme(panel.grid =
element_blank(), # Remove gridlines panel.background = element_blank())
Plot.GMT1.1
my_sum1.2 <- Co.cultivation.expo.2 %>% group_by(Bacteria.strain) %>% summarise(
n=n(), mean=mean(Days.to.germinate), sd=sd(Days.to.germinate) ) %>% mutate(
se=sd/sqrt(n)) %>% mutate( ic=se * qt((1-0.05)/2 + .5, n-1))
```

```
my_sum1.2$Bacteria.strain <- fct_relevel(my_sum1.2$Bacteria.strain, "Control")
my_sum1.2$Bacteria.strain <- as.factor(my_sum1.2$Bacteria.strain)
my_sum1.2$Bacteria.strain <- fct_relevel(my_sum1.2$Bacteria.strain, "Control")
```
Standard deviation
```
Plot.GMT1.2 <- ggplot(my_sum1.2) + geom_bar( aes(x=Bacteria.strain, y=mean),
stat="identity", fill="grey", alpha=0.5) + geom_errorbar( aes(x=Bacteria.strain, ymin=mean-
sd, ymax=mean+sd), width=0.3, colour="black", alpha=0.9, size=0.5) + theme(axis.text.x =
element_blank(), axis.title.x = element_blank()) + labs(y = "GMt")+ theme(panel.grid =
element_blank(), # Remove gridlines panel.background = element_blank())
Plot.GMT1.2
```
Files are Co.cultivation expo 1/2, CocoBact 1/2, Media.Type 1/2
```
########### For Mean cot length at the end of experiment
my_sum2.1 <- Co.cultivation.expo.1 %>% group_by(Bacteria.strain) %>% summarise(
n=n(), mean=mean(Cotalidon.length), sd=sd(Cotalidon.length) ) %>% mutate( se=sd/sqrt(n))
%>% mutate( ic=se * qt((1-0.05)/2 + .5, n-1))
```
Standard deviation
```
ggplot(my_sum2.1) + geom_bar( aes(x=Bacteria.strain, y=mean), stat="identity", fill="grey",
alpha=0.5) + geom_errorbar( aes(x=Bacteria.strain, ymin=mean-sd, ymax=mean+sd),
width=0.3, colour="black", alpha=0.9, size=0.5) + ggtitle("using standard deviation") +
theme(axis.text.x = element_text(angle = 45, hjust = 1)) View(my_sum2.1)
```
combine the plots using https://r-charts.com/ggplot2/combining-plots/
```
my_sum2.1$Bacteria.strain <- fct_relevel(my_sum2.1$Bacteria.strain, "Control")
my_sum2.1$Bacteria.strain <- as.factor(my_sum2.1$Bacteria.strain)
my_sum2.1$Bacteria.strain <- fct_relevel(my_sum2.1$Bacteria.strain, "Control")
```
Standard deviation
```
Plot.cot1 <- ggplot(my_sum2.1) + geom_bar( aes(x=Bacteria.strain, y=mean),
stat="identity", fill="grey", alpha=0.5) + geom_errorbar( aes(x=Bacteria.strain, ymin=mean-
sd, ymax=mean+sd), width=0.3, colour="black", alpha=0.9, size=0.5) + theme(axis.text.x =
element_blank(), axis.title.x = element_blank()) + labs(y = "Cotylidon length (cm)")+
theme(panel.grid = element_blank(), # Remove gridlines panel.background =
element_blank())
Plot.cot1
#####################
my_sum2.2 <- Co.cultivation.expo.2 %>% group_by(Bacteria.strain) %>% summarise(
n=n(), mean=mean(Cotalidon.length), sd=sd(Cotalidon.length) ) %>% mutate( se=sd/sqrt(n))
%>% mutate( ic=se * qt((1-0.05)/2 + .5, n-1))
```
Standard deviation
```
ggplot(my_sum2.2) + geom_bar( aes(x=Bacteria.strain, y=mean), stat="identity", fill="grey",
alpha=0.5) + geom_errorbar( aes(x=Bacteria.strain, ymin=mean-sd, ymax=mean+sd),
width=0.3, colour="black", alpha=0.9, size=0.5) + ggtitle("using standard deviation") +
theme(axis.text.x = element_text(angle = 45, hjust = 1))
```
combine the plots using https://r-charts.com/ggplot2/combining-plots/
```
my_sum2.2$Bacteria.strain <- as.factor(my_sum2.2$Bacteria.strain)
my_sum2.2$Bacteria.strain <- fct_relevel(my_sum2.2$Bacteria.strain, "Control")
```
Standard deviation
```
Plot.cot2 <- ggplot(my_sum2.2) + geom_bar( aes(x=Bacteria.strain, y=mean),
stat="identity", fill="grey", alpha=0.5) + geom_errorbar( aes(x=Bacteria.strain, ymin=mean-
sd, ymax=mean+sd), width=0.3, colour="black", alpha=0.9, size=0.5) + theme(axis.text.x =
element_blank(), axis.title.x = element_blank()) + labs(y = "Cotylidon length (cm)")+
```

```
ylim(0,10)+ theme(panel.grid = element_blank(), # Remove gridlines panel.background =
element_blank())
Plot.cot2 #### Mortality plot######
my_sum3.1 <- Co.cultivation.expo.1 %>% group_by(Bacteria.strain) %>% summarise(
n=n(), mean=mean(Mortality), sd=sd(Mortality) ) %>% mutate( se=sd/sqrt(n)) %>% mutate(
ic=se * qt((1-0.05)/2 + .5, n-1))
my_sum3.1$Bacteria.strain <- fct_relevel(my_sum3.1$Bacteria.strain, "Control")
my_sum3.1$Bacteria.strain <- factor(my_sum3.1$Bacteria.strain)
Standard deviation
Plot.mortality1 <- ggplot(my_sum3.1) + geom_bar( aes(x=Bacteria.strain, y=mean),
stat="identity", fill="grey", alpha=0.5, position = "dodge") + theme(axis.text.x =
element_text(angle = 90, hjust = 1, size = 12))+ labs(y = "Mortality (%)")+ theme(panel.grid
= element_blank(), # Remove gridlines panel.background = element_blank())
Plot.mortality1
####################################
my_sum3.2 <- Co.cultivation.expo.2 %>% group_by(Bacteria.strain) %>% summarise(
n=n(), mean=mean(Mortality), sd=sd(Mortality) ) %>% mutate( se=sd/sqrt(n)) %>% mutate(
ic=se * qt((1-0.05)/2 + .5, n-1))
my_sum3.2$Bacteria.strain <- fct_relevel(my_sum3.2$Bacteria.strain, "Control")
print(my_sum3.2)
Standard deviation
Plot.mortality2 <- ggplot(my_sum3.2) + geom_bar( aes(x=Bacteria.strain, y=mean),
stat="identity", fill="grey", alpha=0.5) + theme(axis.text.x = element_text(angle = 90, hjust =
1, size = 12))+ labs(y = "Mortality (%)")+ theme( axis.text.x = element_text(angle = 90, hjust
= 1, size = 12, face = "italic"), # x-axis labels italic axis.text.y = element_text(face = "italic"),
# y-axis labels italic panel.grid = element_blank(), panel.background = element_blank() ) +
labs(y = "Mortality (%)")
Plot.mortality2
Ensure Bacteria.strain is a factor and reorder
my_sum3.2$Bacteria.strain <- factor(my_sum3.2$Bacteria.strain)
my_sum3.2$Bacteria.strain <- fct_relevel(my_sum3.2$Bacteria.strain, "Control")
Create bar plot with reordered x-axis
Plot.mortality2 <- ggplot(my_sum3.2, aes(x = Bacteria.strain, y = mean)) + geom_bar(stat =
"identity", fill = "grey", alpha = 0.5) + theme(axis.text.x = element_text(angle = 90, hjust = 1,
size = 12)) + labs(y = "Mortality (%)") + theme(panel.grid = element_blank(), # Remove
gridlines panel.background = element_blank()+ axis.title.x = element_text(face = "italic"),)
Plot.mortality2 <- ggplot(my_sum3.2, aes(x = Bacteria.strain, y = mean)) + geom_bar(stat =
"identity", fill = "grey", alpha = 0.5) + theme( axis.text.x = element_text(angle = 90, hjust =
1, size = 12, face = "italic"), # x-axis labels italic axis.text.y = element_text(face = "italic"), #
y-axis labels italic panel.grid = element_blank(), panel.background = element_blank() ) +
labs(y = "Mortality (%)")
Display the plot
Plot.mortality2
#############################################
library(dplyr) my_sum3.1$mean <- my_sum3.1$mean/10 View(my_sum3.1)
my_sum3.1$Bacteria.strain <- as.factor(my_sum3.1$Bacteria.strain)
my_sum3.1$Bacteria.strain <- fct_relevel(my_sum3.1$Bacteria.strain, "Control ")
levels(my_sum3.1$Bacteria.strain)
Create bar plot with reordered x-axis
```

```
Plot.mortality1 <- ggplot(my_sum3.1, aes(x = Bacteria.strain, y = mean)) + geom_bar(stat =
"identity", fill = "grey", alpha = 0.5) + theme(axis.text.x = element_text(angle = 90, hjust = 1,
size = 12)) + labs(y = "Mortality (%)") + theme( axis.text.x = element_text(angle = 90, hjust
= 1, size = 12, face = "italic"), # x-axis labels italic axis.text.y = element_text(face = "italic"),
# y-axis labels italic panel.grid = element_blank(), panel.background = element_blank() ) +
labs(y = "Mortality (%)")
Display the plot
Plot.mortality1
levels(my_sum3.1$Bacteria.strain)
install.packages("patchwork") install.packages("crayon")
library(patchwork)
Plot.cot1 / Plot.GMT1.1 / Plot.mortality1
Plot.cot2 / Plot.GMT1.2 / Plot.mortality2

Clean_HMMRMSTRsubstrate <- HMMRMSTRsubstrate%>% filter_all(all_vars(. !="-"))
subset_data_artica <- Clean_HMMRMSTRsubstrate %>% filter(Species %in%
c("Psychromonas arctica"))
gart <- ggplot(subset_data_artica, aes(Substrate)) gart + geom_bar(aes(fill =
dbCAN.Subfam)) + scale_color_brewer(palette = "Set1")+ ylim(0,20)+ labs(title = "", y =
"Cazyme subfamily count")+ theme(axis.text.x = element_text(angle = 90, vjust = 0.5,
hjust=1, size = 12))+ theme(panel.grid = element_blank(), # Remove gridlines
panel.background = element_blank())
################################################ subset_data_titanicae <-
Clean_HMMRMSTRsubstrate %>% filter(Species %in% c("Vreelandella titanicae"))
gtit <- ggplot(subset_data_titanicae, aes(Substrate)) gtit + geom_bar(aes(fill =
dbCAN.Subfam)) + scale_color_brewer(palette = "Set1")+ ylim(0,20)+ labs(title = "", y =
"Cazyme subfamily count")+ theme(axis.text.x = element_text(angle = 90, vjust = 0.5,
hjust=1, size = 12))+ theme(panel.grid = element_blank(), # Remove gridlines
panel.background = element_blank())
################################################## subset_data_fulg <-
Clean_HMMRMSTRsubstrate %>% filter(Species %in% c("Pseudoalteromonas fuliginea"))
gfulg <- ggplot(subset_data_fulg, aes(Substrate)) gfulg + geom_bar(aes(fill =
dbCAN.Subfam)) + scale_color_brewer(palette = "Set1")+ ylim(0,20)+ labs(title = "", y =
"Cazyme subfamily count")+ theme(axis.text.x = element_text(angle = 90, vjust = 0.5,
hjust=1, size = 12))+ theme(panel.grid = element_blank(), # Remove gridlines
panel.background = element_blank())
##################################################
subset_data_para <- Clean_HMMRMSTRsubstrate %>% filter(Species %in% c("Variovorax
paradoxus"))
gpara <- ggplot(subset_data_para, aes(Substrate)) gpara + geom_bar(aes(fill =
dbCAN.Subfam)) + scale_color_brewer(palette = "Set1")+ ylim(0,20)+ labs(title = "", y =
"Cazyme subfamily count")+ theme(axis.text.x = element_text(angle = 90, vjust = 0.5,
hjust=1, size = 12))+ theme(panel.grid = element_blank(), # Remove gridlines
panel.background = element_blank())
ggplot(CAZYME_melted, aes(x = Species, y = X, fill = number)) + geom_tile() + # Creates
heatmap tiles geom_text(aes(label = number), size = 3) + # Add numbers inside tiles
scale_fill_gradient(low = "white", high = "blue") + # Color scale labs( title = "", x =
"Species", y = "Cazyme family", fill = "Quantity" ) + theme_minimal() + # Clean theme
theme( axis.text.x = element_text( angle = 90, hjust = 0.5, face = "italic" )
test_result <- fisher.test(data_matrix) print(test_result)
```

```
sea_vs_agar <- data_matrix[c("Seawater","Agar"), ] sea_vs_sed <-
data_matrix[c("Seawater","Sediment"), ] agar_vs_sed <- data_matrix[c("Agar","Sediment"),
]
test_sea_agar <- fisher.test(sea_vs_agar) test_sea_sed <- fisher.test(sea_vs_sed)
test_agar_sed <- fisher.test(agar_vs_sed)
test_sea_agar test_sea_sed test_agar_sed

df <- read.csv("C:/Users/Gilbert Jones/Downloads/Colony counts.csv",
stringsAsFactors=TRUE)
Check the first few rows
head(df) print(df)
Summary statistics by Type and Growth Media
summary_table <- df %>% group_by(Type, Growth.media) %>% summarise( Mean_CFU =
mean(Count, na.rm = TRUE), SD_CFU = sd(Count, na.rm = TRUE), Min_CFU =
min(Count, na.rm = TRUE), Max_CFU = max(Count, na.rm = TRUE), .groups = "drop" )
Print summary table
print(summary_table)
ggplot(df, aes(x = Type, y = Count, fill = Growth.media)) + geom_boxplot() +
scale_y_log10(sec.axis = sec_axis(~., name = "CFU Count/g (log scale)")) + # Log scale to
handle large differences in CFU counts labs(title = "", x = "Sample Type", y = "CFU
Count/seed (log scale)") + theme_minimal()+ theme(panel.grid = element_blank(), # Remove
gridlines panel.background = element_blank())
mean_CFU <- df %>% group_by(Type, Growth.media) %>% summarise(Mean_CFU =
mean(Count, na.rm = TRUE), .groups = "drop")
Create bar plot
ggplot(mean_CFU, aes(x = Type, y = Mean_CFU, fill = Growth.media)) + geom_bar(stat =
"identity", position = "dodge") + scale_y_log10() + # Log scale for better visualization
labs(title = "", x = "Sample Type", y = "Mean CFU Count/g (log scale)") +
theme_minimal()+ theme(panel.grid = element_blank(), # Remove gridlines
panel.background = element_blank())
df <- data.frame( Type = c("Sediment", "Sediment", "Sediment", "Sediment", "Sediment",
"Sediment", "Seed", "Seed", "Seed", "Seed", "Seed", "Seed"), Sample = rep(1:3, each = 2,
times = 2), Growth.media = rep(c("MB", "F2"), times = 6), Count = c(135000, 55000,
120000, 1190000, 5900, 350000, 100, 80000, 0, 140000, 0, 490000) )
Optional: log-transform the data to stabilize variance
df$logCount <- log10(df$Count + 1) # add 1 to avoid log(0)
1. T-test for SEED samples
seed_data <- subset(df, Type == "Seed") t.test(logCount ~ Growth.media, data = seed_data)
2. T-test for SEDIMENT samples
sediment_data <- subset(df, Type == "Sediment") t.test(logCount ~ Growth.media, data =
sediment_data)
############ BCG pie charts##############
library(ggplot2) library(dplyr) library(readr)
Load your long-format CSV file
bgc_data <- read.csv("C:/Users/Gilbert Jones/Downloads/BCG_long_format.csv")
View(bgc_data)
Create pie charts for each species
bgc_data %>% ggplot(aes(x = "", y = Count, fill = Type)) + geom_col(width = 1) +
coord_polar(theta = "y") + facet_wrap(~ Species, scales = "free") + labs(title = "", fill =
```

"BGC Type") + theme_void() + theme(strip.text = element_text(size = 12, face = "bold"), plot.title = element_text(size = 16, face = "bold", hjust = 0.5))

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9 Expenditure

Item	Supplier	specs	Price
Corning Stripettor Ultra Pipette Filler - Promotional Offer plus serological pipettes	SLS		245
Guillard′s (F/2) Marine Water Enrichment Solution	Sigma-Aldrich	500 mL	63.2
Sucrose	Sigma-Aldrich	500 g	34.8
Brady label	SLS		87.85
X50 6-Well Culture Plate, PS, Clear, TC-Treated, Flat Bottom, Lid, Sterile, Individually Wrapped	Fisher Scientific		134.35
Glycerol	Fisher Scientific		16.28
GENEJET PCR PURIF 50PREP	Fisher Scientific		68.07
6 spray bottles	SLS		28.68
4 Select Petri Dish TV 90 mm	SLS		153.72
Wizard(R) HMW DNA Extraction Kit, 50 preps	Promega		223
PCR reactions		x8	40
Dreamtaq (200 reactions)			94.65
1.5 ml TubeOne® Microcentrifuge Tube			21.76
Gloves			49
Pippette tips, 960? Eg 4 x960			50
Preparation Tube, 5.0 ml, Conical, Natural / 250			42.34
Total			1352.7

10. Statement of contributions

Contributor Role	Persons involved
Conceptualization	ECS, JJ, BW
Data Curation	JJ
Formal Analysis	JJ
Funding Acquisition	N/a
Investigation	JJ
Methodology	ECS, JJ, BW
Project Administration	ECS, JJ, BW
Resources	ECS, JJ, TW
Software	JJ
Supervision	ECS
Validation	N/a
Visualization	JJ
Writing – Original Draft	JJ
Writing – Review & editing	ECS, JJ

11. Ethics Approval

Swansea Univèrs"ity

Research Ethics Approval Number: 1 2024 9265 8148

Project Title	Deciphering the seagrass microbiome for advanced ecosystem restoration
Sub+Lleóbv	MB J€GI2R I XINE6."
Full application form link	https://swansea.forms.ethicalreviewmanager.com/ProjectIndex/11266

2. This project may be subject to periodic review by the committee. The approval may be suspended or revoked at any time if there has been a breach of

Science and Engineering Research and Ethics Chair

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12. H&S and Risk Assessments

Swansea University Prifysgol Abertawe | HEALTH & SAFETY IECHYD A DIOGELWCH

Risk Assessment of An Activity Involving Deliberate Work with Pathogenic Microorganisms or Samples with Potential to Harbour Pathogenic Microorganisms

This risk assessment form should be used to assist in the assessment of risks from an activity involving deliberate work with an infectious of harmful biological agent. The aim of the assessment is to identify those at risk from infection or other harm and the measures required to eliminate or control the risks to human health and the environment to an acceptable level.

Section 1: General Information

1.1 Principal Investigator/ Academic Supervisor:

Name	Faculty
Eva Sonnenschein	Science and Engineering
Email	Date
[redacted]	24/07/2024

1.2 Premises where this work will be carried out:

Building	Laboratories	Containment level
Wallace	120, 123, 124, 131a	1

Section 2: Project Information

2.1 Person undertaking this risk assessment (if different from above):

Name	Faculty
Joseph Jones	Science and Engineering

2.2 Project title:

Deciphering the seagrass microbiome for advanced ecosystem restoration

2.3 Project Reference Number:
Suggested format YYMMDD, PI initials, BIO (number if more than one in a month)

240710_MME_ECS

2.3 Is this proposal an extension of a previously approved project?

Previously approved If yes, please complete the form emphasising the connection between the original project and this application.	☐ Yes ☒ No
Previous reference number	

2.4 Brief overview of the work (in layman's terms):

Please describe the project, detailing aims and objectives, significance, and outcomes, indicating how the biological agent will help to achieve the objectives of the project. This description should contain enough detail to help a non-specialist to understand the project.	
Non hazardous strains will help describe the seagrass microbiome. Isolates will be grown on agar plates and in liquid media, for further genome sequence testing. This will contribute to the understanding of the seagrass microbiome, and determine whether it plays a role in the germination success of seeds.	
2.5 Is this biological agent going to form part of an undergraduate practical class?	☐ Yes ☒ No
2.6 Faculty Contacts:	
Health, Safety and Resilience Advisor	Lorraine Wild and Will Finn-Lewis
Biological Safety Officer	Dr Tamsyn Uren Webster

Section 3: Identification of Biological Hazards

3.1 List microorganisms deliberately used/ or pathogenic microorganisms that potentially can be present in the sample(s):

Name of microorganism	*Chryseobacterium indoltheticum*	*Psychromonas arctica*	*Vibrio hispanicus*
Identified as human pathogen on ACDP list[1]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
If not on ACDP list, is there any evidence to support the microorganism may present a risk to human health	No	No	No
Normal routes of human infection	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen

Multiplicity of infection if known (i.e., number of organisms required to establish an infection)	Not a human pathogen	Not a human pathogen	Not a human pathogen .
Consequence of infection to humans	Not known	Not known	Not known
Is the microorganism a specified animal pathogen (SAPO[2])	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state SAPO hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
Detail of any other harm the microorganism may pose to the environment? e.g., harmful to plants, insects, etc.	No known risk	No known risk	No known risk
Consequence of spread in environment	No known consequences	No known consequences	No known consequences
Route of transmission for environmental pathogens (including animals)	Not known	Not known	Not known
Any additional risk to health/ environment e.g. Hyper virulence, multiple	N/A	N/A	N/A

antibiotic resistance			
Listed on Schedule 5[3]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
How likely is that Cat 3 and Cat 4 biological agents will be present in the material/ sample to be handled?	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present
Will you intentionally isolate, propagate or otherwise increase the risk of the above mentioned (if any) CAT3 and CAT4 biological agents?	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No

[1] ACDP Approved List of (Human) Pathogens
[2] *SAPO Pathogens*
[3] Schedule 5 Pathogens on the Anti-terrorism & Security

Section 3: Identification of Biological Hazards

3.1 List microorganisms deliberately used/ or pathogenic microorganisms that potentially can be present in the sample(s):

Name of microorganism	*Colwellia echini*	*Psychromonas marina*	*Marinomonas primoryensis*
Identified as human pathogen on ACDP list[1]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
If not on ACDP list, is there any evidence to support the	No	No	No

microorganism may present a risk to human health			
Normal routes of human infection	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen
Multiplicity of infection if known (i.e., number of organisms required to establish an infection)	Not a human pathogen	Not a human pathogen	Not known
Consequence of infection to humans	Not known	Not known	Not known
Is the microorganism a specified animal pathogen (SAPO[2])	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state SAPO hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
Detail of any other harm the microorganism may pose to the environment? e.g., harmful to plants, insects, etc.	Not known	Not known	Not known
Consequence of spread in environment	None	None	None

Route of transmission for environmental pathogens (including animals)	Not known	Not known	Not known
Any additional risk to health/ environment e.g. Hyper virulence, multiple antibiotic resistance	N/A	N/A	N/A
Listed on Schedule 5[3]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
How likely is that Cat 3 and Cat 4 biological agents will be present in the material/ sample to be handled?	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present
Will you intentionally isolate, propagate or otherwise increase the risk of the above mentioned (if any) CAT3 and CAT4 biological agents?	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No

[1] ACDP Approved List of (Human) Pathogens
[2] *SAPO Pathogens*
[3] Schedule 5 Pathogens on the Anti-terrorism & Security

Section 3: Identification of Biological Hazards

3.1 List microorganisms deliberately used/ or pathogenic microorganisms that potentially can be present in the sample(s):

Name of microorganism	*Pseudoalteromonas fuliginea*	*Psychromonas arctica*	*Variovorax paradoxus*
Identified as human pathogen on ACDP list[1]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
If not on ACDP list, is there any evidence to support the microorganism may present a risk to human health	No	No	No
Normal routes of human infection	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen
Multiplicity of infection if known (i.e., number of organisms required to establish an infection)	Not a human pathogen	Not a human pathogen	Not a human pathogen .
Consequence of infection to humans	Not known	Not known	Not known
Is the microorganism a specified animal pathogen (SAPO[2])	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No

If yes, please state SAPO hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
Detail of any other harm the microorganism may pose to the environment? e.g., harmful to plants, insects, etc.	No known risk	No known risk	No known risk
Consequence of spread in environment	No known consequences	No known consequences	No known consequences
Route of transmission for environmental pathogens (including animals)	Not known	Not known	Not known
Any additional risk to health/ environment e.g. Hyper virulence, multiple antibiotic resistance	N/A	N/A	N/A
Listed on Schedule 5[3]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
How likely is that Cat 3 and Cat 4 biological agents will be present in the material/ sample to be handled?	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present
Will you intentionally isolate, propagate	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No

or otherwise increase the risk of the above mentioned (if any) CAT3 and CAT4 biological agents?			

[1] ACDP Approved List of (Human) Pathogens
[2] *SAPO Pathogens*
[3] Schedule 5 Pathogens on the Anti-terrorism & Security

Section 3: Identification of Biological Hazards

3.1 List microorganisms deliberately used/ or pathogenic microorganisms that potentially can be present in the sample(s):

Name of microorganism	*Pseudoalteromonas spiralis*	*Lysobacter luteus*	*Variovorax paradoxus*
Identified as human pathogen on ACDP list[1]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
If not on ACDP list, is there any evidence to support the microorganism may present a risk to human health	No	No	No
Normal routes of human infection	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen
Multiplicity of infection if known (i.e., number of organisms required to	Not a human pathogen	Not a human pathogen	Not a human pathogen .

establish an infection)			
Consequence of infection to humans	Not known	Not known	Not known
Is the microorganism a specified animal pathogen (SAPO[2])	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state SAPO hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
Detail of any other harm the microorganism may pose to the environment? e.g., harmful to plants, insects, etc.	No known risk	No known risk	No known risk
Consequence of spread in environment	No known consequences	No known consequences	No known consequences
Route of transmission for environmental pathogens (including animals)	Not known	Not known	Not known
Any additional risk to health/ environment e.g. Hyper virulence, multiple antibiotic resistance	N/A	N/A	N/A

Listed on Schedule 5[3]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
How likely is that Cat 3 and Cat 4 biological agents will be present in the material/ sample to be handled?	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present
Will you intentionally isolate, propagate or otherwise increase the risk of the above mentioned (if any) CAT3 and CAT4 biological agents?	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No

[1] ACDP Approved List of (Human) Pathogens
[2] *SAPO Pathogens*
[3] Schedule 5 Pathogens on the Anti-terrorism & Security

Section 3: Identification of Biological Hazards

3.1 List microorganisms deliberately used/ or pathogenic microorganisms that potentially can be present in the sample(s):

Name of microorganism	*Pseudoalteromonas spiralis*	*Lysobacter luteus*	*Alkalicoccobacillus plakortidis*
Identified as human pathogen on ACDP list[1]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
If not on ACDP list, is there any evidence to support the microorganism may present a	No	No	No

risk to human health			
Normal routes of human infection	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen
Multiplicity of infection if known (i.e., number of organisms required to establish an infection)	Not a human pathogen	Not a human pathogen	Not a human pathogen .
Consequence of infection to humans	Not known	Not known	Not known
Is the microorganism a specified animal pathogen (SAPO[2])	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state SAPO hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
Detail of any other harm the microorganism may pose to the environment? e.g., harmful to plants, insects, etc.	No known risk	No known risk	No known risk
Consequence of spread in environment	No known consequences	No known consequences	No known consequences

Route of transmission for environmental pathogens (including animals)	Not known	Not known	Not known
Any additional risk to health/ environment e.g. Hyper virulence, multiple antibiotic resistance	N/A	N/A	N/A
Listed on Schedule 5[3]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
How likely is that Cat 3 and Cat 4 biological agents will be present in the material/ sample to be handled?	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present
Will you intentionally isolate, propagate or otherwise increase the risk of the above mentioned (if any) CAT3 and CAT4 biological agents?	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No

[1] ACDP Approved List of (Human) Pathogens
[2] *SAPO Pathogens*
[3] Schedule 5 Pathogens on the Anti-terrorism & Security

Section 3: Identification of Biological Hazards

3.1 List microorganisms deliberately used/ or pathogenic microorganisms that potentially can be present in the sample(s):

Name of microorganism	*Celeribacter baekdonensis*	*Rhodococcus cerastii*	*Rhodococcus yunnanensis*
Identified as human pathogen on ACDP list[1]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
If not on ACDP list, is there any evidence to support the microorganism may present a risk to human health	No	No	No
Normal routes of human infection	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen
Multiplicity of infection if known (i.e., number of organisms required to establish an infection)	Not a human pathogen	Not a human pathogen	Not a human pathogen.
Consequence of infection to humans	Not known	Not known	Not known
Is the microorganism a specified animal pathogen (SAPO[2])	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state SAPO hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4

Detail of any other harm the microorganism may pose to the environment? e.g., harmful to plants, insects, etc.	No known risk	No known risk	No known risk
Consequence of spread in environment	No known consequences	No known consequences	No known consequences
Route of transmission for environmental pathogens (including animals)	Not known	Not known	Not known
Any additional risk to health/ environment e.g. Hyper virulence, multiple antibiotic resistance	N/A	N/A	N/A
Listed on Schedule 5[3]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
How likely is that Cat 3 and Cat 4 biological agents will be present in the material/ sample to be handled?	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present
Will you intentionally isolate, propagate or otherwise increase the risk of the above mentioned (if any)	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No

CAT3 and CAT4 biological agents?			

[1] ACDP Approved List of (Human) Pathogens
[2] *SAPO Pathogens*
[3] Schedule 5 Pathogens on the Anti-terrorism & Security

Section 3: Identification of Biological Hazards

3.1 List microorganisms deliberately used/ or pathogenic microorganisms that potentially can be present in the sample(s):

Name of microorganism	*Vreelandella titanicae*		
Identified as human pathogen on ACDP list[1]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☐ No
If yes, please state hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
If not on ACDP list, is there any evidence to support the microorganism may present a risk to human health	No		
Normal routes of human infection	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen	☐ Inhalation ☐ Oral/ ingestion ☐ Mucocutaneous ☐ Percutaneous ☐ Via vector (e.g., insect) ☐ Allergen
Multiplicity of infection if known (i.e., number of organisms required to establish an infection)	Not a human pathogen	N/a	

Consequence of infection to humans	Not known	Vibriosis	Not known
Is the microorganism a specified animal pathogen (SAPO[2])	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No
If yes, please state SAPO hazard group	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4	☐ 2 ☐ 3 ☐ 4
Detail of any other harm the microorganism may pose to the environment? e.g., harmful to plants, insects, etc.	No known risk	No known risk	
Consequence of spread in environment	No known consequences	No known consequences	
Route of transmission for environmental pathogens (including animals)	Not known	Ingestion	
Any additional risk to health/ environment e.g. Hyper virulence, multiple antibiotic resistance	N/A	None	
Listed on Schedule 5[3]	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☐ No

How likely is that Cat 3 and Cat 4 biological agents will be present in the material/ sample to be handled?	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☒ Unlikely present	☐ Definitely present ☐ Likely present ☐ Unlikely present
Will you intentionally isolate, propagate or otherwise increase the risk of the above mentioned (if any) CAT3 and CAT4 biological agents?	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☐ No

[1] ACDP Approved List of (Human) Pathogens
[2] *SAPO Pathogens*
[3] Schedule 5 Pathogens on the Anti-terrorism & Security

Section 4: Experimental Procedures

4.1 Description of experimental procedures:

Cultivation of bacteria in liquid culture and then plating on agar medium.
Cocultivation germination experiments. Germination experiments

4.2 Quantities used and frequency of use:

This information will enable you to determine the likelihood of exposure and therefore the risks from this particular activity. Please indicate maximum culture volumes at any time shown as multiples of flask volumes to give an idea of scale.

Max. volume per culture/ sample	5 ml	Max. volume per experiment	0.5 ml
Frequency of experiments		Once to isolate and 16s RNA sequence	

Section 5: Measures to Prevent or Control Exposure

5.1 Preventing exposure

a) Could a less hazardous substance (or form of the substance) be used instead? If it can, then it should be used, or justification be given here why it is not being used. COSHH requires substitution with less hazardous materials wherever possible, but there may be good reasons for not using them.

Vibrio alginolyticus unused in germination experiments.

5.2 Controlling Exposure

b) Containment Level - what containment level is required for this work with regard to COSHH/ SAPO?		
☒ 1	☐ 2	☐ 3

c) CL3 only – application for derogation from the following controls (list if relevant and justify):	
d) Will the work be segregated from others not involved in the work and if not, how will they be informed of the hazards?	

5.3 Engineering Controls (Containment & Ventilation)	
a) Is a microbial safety cabinet (or isolator for in vivo work) required? These must be used for activities generating potentially infectious aerosols or splashes.	☒ Yes ☐ No
If yes, please state which class and describe underneath what processes will use it:	Class: ☐ I ☐ II ☐ III Unsure
If required, what processes require its use?	
b) Specify other local ventilation control measures considered appropriate (e.g., downdraft table, isolator):	
c) Will centrifugation be used?	☒ Yes ☐ No
If yes, will buckets and rotors be sealed?	☐ Yes ☒ No
If yes, where will buckets or rotors be opened?	Biosafety Cabinet
If yes, how will spillages in the centrifuge be dealt with?	Disinfection with BioCleanse or 70% ethanol and blue roll
d) Will incubators be used?	☒ Yes ☐ No
If yes, what type (e.g., shaking)?	Shaking and temperature controlled.
If yes, how will spillages in the incubator be dealt with?	Disinfection with Biocleanse or 70% ethanol and blue roll
e) Will sharps be used:	☐ Yes ☒ No
If yes, list and justify their use:	
f) Will animals be deliberately infected with these biological agents?	☐ Yes ☒ No
g) Do you require a licence/ permit? https://staff.swansea.ac.uk/media/tgn002-abps-and-import-permits.pdf	☐ Yes ☒ No
h) If yes, describe the procedure, control measures and whether shedding of infectious agents by animals is expected?	

5.4 Personal Protective Equipment (PPE):			
Lab coat	Gloves	Eye or face (specify)	Other
☒ Yes	☒ Yes ☐ No	☐ Yes ☒ No	☐ Yes ☒ No
Details:	Details:	Details:	Details:

5.5 Transportation	
a) How will viable material be transported within the laboratory?	In covered flasks and beakers with liquid media. On covered agar plates.
b) How will viable material be transported locally outside the laboratory?	In covered flasks and beakers with liquid media. On covered agar plates. All material will be transported within a covered secondary container to prevent spillages.
c) Will viable material be shipped anywhere (off campus)?	☐ Yes ☒ No
If yes, what will be shipped?	
If yes, how will this be shipped (e.g., Category A, Category B, Exempt, Non-hazardous)?	

5.6 Waste Disposal Procedures: (Disinfectants, concentrations, exposure times, autoclaving procedures, incinerator procedures, include any animal related wastes.)		
Waste	Decontamination method (include details on efficacy)	Disposal route e.g., drain/ incineration/ landfill
Liquid waste	Autoclaving to kill the bacteria.	Drain.
Solid waste	Autoclaving to kill the bacteria.	Autoclaved waste.
Sharp waste	In yellow bins as non-hazardous waste.	In yellow bins.

5.7 Emergency Procedures

Spillages

- If covered by local rules/ standard operating procedure, please attach.
- If not covered by local rules/ standard operating procedure. Remember to take into account route of exposure

a) Inside primary containment (if relevant e.g., MSC, isolator):	Disinfection with Biocleanse or similar 70% Ethanol and blue roll.
b) Outside primary containment but within the laboratory (secondary containment):	Disinfection with Biocleanse or similar 70% Ethanol and blue roll.

c) Outside secondary containment (if relevant):	Disinfection with Biocleanse or similar 70% Ethanol and blue roll.
d) Other procedures (e.g., first aid following any accidental exposure, needle stick, etc.):	N/A

Section 6: Personnel and Health Issues

6.1 Vaccination

For ACDP 2 or above human pathogens Is an effective vaccination available for any of the pathogens associated with this work?	N/A

6.2 Is health surveillance/ health clearance required?

Staff and postgraduate research students	☐ Yes ☒ No
Taught students (undergraduate and MSc)	☒ Yes ☐ No (Initial health clearance only)

6.3 Identify any groups of workers who may be at increased risk from this material:

Anyone who might have compromised resistance to disease for any reason should seek advice from the University Occupational Health Service regarding the need for additional precautions. (For example, pregnant workers, young persons under 18, disabled workers, those with pre-existing disease that increases susceptibility).	

6.4 Information, Instruction, and Training

Describe the training that will be given to all those affected (directly or indirectly) by the work activity.	PG students and staff are required to complete mandatory Canvas Health and Safety course, individual lab inductions and risk assessment forms.

Section 7: Declaration and Approval

7.1 Principal Investigator:

I the undersigned:

- Confirm that all information contained in this assessment is correct and up to date.
- Will ensure that suitable and sufficient instruction, information, and supervision is provided to all individuals working on the activity.
- Will ensure that no work will be carried out until this assessment has been completed and approved, and that all necessary control measures are in place.
- Will ensure that all information contained in this assessment will remain correct and up to date and re-submit for approval if any significant changes occur.
- Work will only be undertaken in appropriate facilities.

Name	Signature	Date

7.2 Approval on behalf of the Faculty:

- Approval of Hazard Group 1 only.
- I support the presentation of this proposal to the Biological Hazards and Genetic Modified Organisms Sub-Committee (for ACDP Hazard Group 2-4, SAPO Hazard Group 2-4 and organisms listed on schedule 5).

The person supporting this proposal must not be involved in the project being proposed.

Faculty BSO	Name	
	Signature	
	Date	
University BSO	Name	
	Signature	
	Date	

7.3 Approval on behalf of the University:

- The risk assessment has been reviewed and approved by the Biological Hazards and GMO Sub-Committee.
- Approval of ACDP Hazard Group 2-4, SAPO Hazard Group 2-4 and organisms listed on schedule 5.

Chair of Biological Hazards and GMO Sub-Committee	Name	
	Signature	
	Date	

Section 8: List of Workers Under This Project

List any persons who will be working on this project. They must have access to this risk assessment and other associated risk assessments. Those listed should sign and date to confirm they have read understand the risk assessment.

Full Name	Worker Type					Signature and date
	Staff	PG Research	PG Taught	UG	Other	
Eva Sonnenschein	☒	☐	☐	☐	☐ Details	[redacted] 07/10/24
Joseph Jones	☐	☒	☐	☐	☐ Details	[redacted] 05/10/24
	☐	☐	☐	☐	☐ Details	
	☐	☐	☐	☐	☐ Details	

	☐	☐	☐	☐	☐ Details	
	☐	☐	☐	☐	☐ Details	

This can also be completed manually and kept as a hard copy in the laboratory – copies must be available for review by Biological Safety Officer/ Biological Safety Advisor.

V. 2023

Risk Assessment for Teaching, Administration and Research Activities

Swansea University; FSE: Biosciences

Name, Signature Joe Jones JJ **, date 12/04/2024**

Supervisor* Dr Eva Sonnenschein Signature [redacted]
..... **date.....** 07/10/24

Activity title Microbial isolation and cultivation
(room no.) W123, W131A.....
(* the supervisor for all HEFCW funded academic and non-academic staff is the HOD)

University Activity Serial # (enter Employee No. or Student No.)..... [redacted]
Start date of activity (cannot predate signature dates) 12/4/24
End date of activity (or 'on going') on going .....

Level of worker (choose from the list below) M.res

UG, MSc, M.Res, M.Phil/PhD, RA/Postdoc, technician, administration, academic staff, visitor, other (state)

Ethics approval number …1 2024 9265 8148

Approval obtained for Biological Hazards and/or GMO Safety Assessment by SU? Yes/not applicable

Approval obtained for use of radioisotopes
not applicable

(If the answer is YES to any of the above, then other specific rules will apply -please seek advice)

Record of specialist training undertaken (see Training record document)

Course	Check appropiate

Summary of protocols used; protocol sheets to be appended and updated as necessary

Protocol Details						Protocol Details					
#	Assessment					#	Assessment				
	1st date	Frequency of re-assessment	Hazard category	Secondary containment level	Exposure potential		1st date	Frequency of re-assessment	Hazard category	Secondary containment level	Exposure potential
1						11					
2						12					
3						13					
4						14					
5						15					
6						16					
7						17					
8						18					
9						19					
10						20					

(Continue on another sheet if necessary)

Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol	**Title: Microbial isolation and cultivation**
Associated Protocols #.....	**Location (**e.g. Boat, Field (give locations, Laboratory, Office, Facility). Identify here risks and control measures for work in this environment, additional to Local Rules: Laboratory

Description:

Plating bacterial cultures on 2 types of agar growth media. Restreaking cultures using inoculation loop.
Full description
Bacterial isolation from seagrass samples · Benchling

CHEMICAL RISK – Summary sheet

A copy of each Chemical COSSH form should be readily available in the lab for use (e.g. in an emergency)

Chemical Name (& Conc.) for chemicals to be used and generated	**GHS symbols** (SH, AT, H, C, Ex, F, O, Env, CG) All that are applicable.	**Skin/Eyes Group** (SA, SB, SC, SD, SE)	**Inhalation Group** (A,B,C,D,E)	**Quantity**	**In use dustiness or volatility**	**Disposal**	**Primary containment & storage**	**Other comments:** **In use factors affecting exposu and special control measures** (e.g. <15 mins duration/ frequenc splash protection only/ hand immersion/ spraying) **Safety/ environmental hazards (H2XX/H4XX)**
Ammonium sulphate	H	SA	A	1 - Small	Low dustiness		Tightly closed. Dry. Storage	Low exposure, measuring quantities for media preperation
Agar	H	SA	B	1 - Small	Medium dustiness	Waste material must be disposed of in accordance with the national and local regulations.	Tightly closed. Dry. Storage	Low exposure, measuring quantities for media preperation

						Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. Notice Directive on waste 2008/98/EC.		
MB/10 nutrients	n/a	SA	B	1 - Small	High dustiness		Tightly closed. Dry. Storage	Low exposure, measuring quantities for media preperation
F/2 Nutrients	n/a	SA	A	1 - Small	Low dustiness		Tightly closed, stored at -20C	Low exposure, measuring quantities for media preperation
Sucrose	n/a	N/A	A	1 - Small	Low dustiness			Low exposure, measuring quantities for media preperation
		Choose an item.	Choose an item.	Choose an item.	Choose an item.			

GHS symbols– SH (serious health hazard), AT (acute toxicity), H (health hazard), C (corrosive), Ex (explosive), F (flammable), O (oxidiser), Env (environment), CG (compressed gas). These should be obtained from chemical SDS documentation. See Appendix (hazard symbols).
Inhalation Group and Skin/Eyes Group- Hazard groups are classified as A/SA (least hazardous) to E/SE (most hazardous). See Appendix for hazard phrases associated with each group. Hazard phrases can be found on chemical SDS documentation.
Dustiness. Low (Pellet- does not break up), Medium (granular or crystalline), High (fine solid or light powder/dust)
Volatility. Low, medium, high, gas. Consider boiling point of liquid and operating temperature.
Disposal e.g. autoclaving of biohazard, SU chemical disposal
Primary containment: e.g. sealed flask, supplied vessel. **Storage:** e.g. secure chemical storage, fridge, freezer, general chemical storage

PROTOCOL RISK MANAGEMENT

Secondary Containment (of protocol): open bench/fume hood/special (highlight/state)

Working Practice – Good Laboratory Practice under local rules PLUS the following: (highlight/state)

Other risks & control measures e.g. pressure, temperature, electrical, mechanical, autoclave, field, boat.

Identify all specific control measures (highlight or delete) - latex/nitrile/heavy gloves; safety glasses, screens; full face mask; dust mask; protective shoes; spillage tray; ear-defenders; other (state)
n.b. details for each chemical are in individual COSSH forms

Justification and controls for any work outside normal hours

Emergency procedures (e.g. first aid, spillage clearance; communication methods)
n.b. full emergency plans for each chemical are detailed in individual COSSH forms

Agarose: Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses. Gloves material: Nitrile rubber Minimum layer thickness: 0.11 mm Break through time: 480 min Material tested:KCL 741 Dermatril® L. Respiratory protection required when dusts are generated, Filter type: Filter type P1.

Spillage of microbial cultures is a risk. Control measure: whilst falcon tube lids cannot be sealed (which would prevent microbial respiration), ensure that they are screwed on loosely. Stop stirrer when handling falcon tubes. Tighten falcon tube lids if transporting between laboratories.

Supervision/training for worker (highlight) **N.B. All relevant training forms (e.g. for specific laboratories) should be attached**

None required Already trained Training required Supervised always

Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker .....Joe Jones, JJ *Name & counter-signature of supervisor.....* *Date.....*	
Date of first reassessment	Frequency of reassessments

Guidance for Completion of Protocol Risk Assessment Form

Note – you are strongly advised to complete electronic versions of this form, enabling you to readily expand and contract sections as required to ensure clarity and adequate documentation. Do **not** delete any sections! Instead, mark inappropriate sections with NA (not applicable) and contract the section to save space on the final printed form.

Protocol - any self-contained procedure. This could be any activities undertaken, be they lab-work, use of equipment, fieldwork or office work. Your complete research/teaching/administration **activity** (e.g. undergraduate project, PhD study, research grant, other) is therefore made up from separate **protocols**. If the protocol is mainly of low hazard, but with one or more hazardous components, consider making the manipulation of the latter a separate protocol and tie them together by completing the "*Associated Protocol*" box. This is because the entire protocol must be conducted under conditions required for the handling of the most hazardous component.

Title - give sufficient detail to make it obvious what the protocol involves.

Location – identify which local rules apply. More than one rule may apply. Then add any additional risks and control measures peculiar to this protocol (e.g. site-specific fieldwork information; use of autoclaves, sonicators; mechanical, electrical hazards). You may also wish to stress any particularly important risks and controls even if indicated in local rules.

Description - give sufficient detail to make it obvious what the protocol involves.

Secondary containment of protocol- detail where the protocol will be performed (refer to Table 4).

Identify other control measures – typically these refer to special protective clothing etc.

Justification and controls for any work outside normal hours – out of hours working is only allowed under special conditions (e.g. 24h sampling, sampling related to tides etc.); convenience is not an acceptable reason.

Emergency procedures - detail how spillages etc. would be handled, including clearance of the laboratory etc. as required. For field work indicate emergency communication and first-aid coverage.

Supervision/training - detail here what special supervision and training is required by the worker named at the bottom of the form. Note that all undergraduates are always considered as research incompetent. First-year PhD students and MSc students are not to be used to supervise the activities of others.

Declaration - both the worker and the supervisor must sign this on the date entered here.

Reassessment - the first reassessment must be undertaken as soon as possible after the first time the protocol has been undertaken in order to identify any unforeseen hazards. After this first reassessment, the protocol should be reassessed every 6-12m, depending on the nature of the chemicals, to take account of changing knowledge concerning the hazardous nature of chemicals. The protocol must be reassessed immediately if new knowledge on the chemical hazards becomes available.

NOTE - standard protocols can be produced for each environment BUT each worker must have their own personalised version, signed by them and their supervisor, and dated. These completed personalised protocols must then be appended to the SU risk assessment form for the Teaching/Research activity belonging to the individual.

CHEMICAL RISK ASSESSMENT

GB CLP hazard pictograms


Explosive (Symbol: exploding bomb)


Hazardous to the environment (Symbol: environment)


Flammable (Symbol: flame)


Health hazard/Hazardous to the ozone layer (Symbol: exclamation mark)


Oxidising (Symbol: flame over circle)


Serious health hazard (Symbol: health hazard)


Corrosive (Symbol: corrosion)


Gas under pressure (Symbol: gas cylinder)


Acute toxicity (Symbol: skull and crossbones)

Hazard group

A	B	C	D	E
H304, H315, H319, H336 and all H-numbers not otherwise listed	H302, H312, H332, H371	H301, H311, H314, H317, H318, H331, H335, H370, H373	H300, H310, H330, H351, H360, H361, H362, H372	H334, H340, H341, H350
Least Hazardous Substances →			More Hazardous Substances	Special cases
Notes: All phrases/hazard statements now considered a risk on or via the skin.				

Hazard phrases associated with hazard groups A-E (chemicals caused harm by inhalation) and hazard groups SA-SE (chemicals causing harm via skin or eye contact). More information available here: https://www.she.stfc.ac.uk/Pages/ChemicalRiskAssessment.pdf

V. 2023

Risk Assessment for Teaching, Administration and Research Activities

Swansea University; FSE: Biosciences

Name Joe Jones **Signature ..... date** 25/03/2024

Supervisor* Eva Sonnenschein .....**Signature** [redacted] **date** 07/10/24

Activity title Agarose gel electrophoresis **Base location (room no.)** Wallace 123, 131A

University Activity Serial # (enter Employee No. or Student No.). [redacted]

Start date of activity (cannot predate signature dates) 25/04/2024

End date of activity (or 'on going') on going

Level of worker (choose from the list below) M.Res

UG, MSc, M.Res, M.Phil/PhD, RA/Postdoc, technician, administration, academic staff, visitor, other (state)

Ethics approval number 1 2024 9265 8148

Approval obtained for Biological Hazards and/or GMO Safety Assessment by SU? Yes/not applicable

Approval obtained for use of radioisotopes
Yes/not applicable

(If the answer is YES to any of the above, then other specific rules will apply -please seek advice)

Record of specialist training undertaken (see Training record document)

Course	Check appropiate

Summary of protocols used; protocol sheets to be appended and updated as necessary

Protocol Details						Protocol Details					
#	Assessment					#	Assessment				
	1st date	Frequency of re-assessment	Hazard category	Secondary containment level	Exposure potential		1st date	Frequency of re-assessment	Hazard category	Secondary containment level	Exposure potential
1						11					
2						12					
3						13					
4						14					
5						15					
6						16					
7						17					
8						18					
9						19					
10						20					

(Continue on another sheet if necessary)

Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #HSEMK01	**Title: Agarose gel electrophoresis**
Associated Protocols #.....	**Location (**e.g. Boat, Field (give locations, Laboratory, Office, Facility). Identify here risks and control measures for work in this environment, additional to Local Rules: Wallace lab 131A

Description:

https://benchling.com/s/prt-hJJiAOFxFOrfma9u76tJ?m=slm-IYUP7ezygAbbWCyhRwAS

CHEMICAL RISK – Summary sheet

A copy of each Chemical COSSH form should be readily available in the lab for use (e.g. in an emergency)

Chemical Name (& Conc.) for chemicals to be used and generated	**GHS symbols** (SH, AT, H, C, Ex, F, O, Env, CG) All that are applicable.	**Skin/Eyes Group** (SA, SB, SC, SD, SE)	**Inhalation Group** (A,B,C,D,E)	**Quantity**	**In use dustiness or volatility**	**Disposal**	**Primary containment & storage**	**Other comments:** **In use factors affecting expo and special control measure** (e.g. <15 mins duration/ freque splash protection only/ hand immersion/ spraying) **Safety/ environmental hazar (H2XX/H4XX)**
Agarose		N/A	N/A	1 - Small	Low dustiness	Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. Notice Directive on waste 2008/98/EC.	Tightly closed. Dry. Storage class (TRGS 510): 11: Combustible Solids	

Tris-acetate-EDTA (TAE) buffer		SC	D	2 - Medium	Choose an item.	Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. Notice Directive on waste 2008/98/EC.	Keep containers tightly closed in a dry, cool and well-ventilated place. Storage class (TRGS 510): 12: Non-flammable liquids in non-flammable packages.	H226, H301, H310, H314, H31 H317, H318, H319, H330, H40 H410, H411
Nucleic acid stain (e.g. SYBR Safe)		N/A	N/A	1 - Small	Choose an item.	SYBR Safe DNA gel stain is not classified as hazardous waste under U.S. Federal regulations (Resource Conservation and Recovery Act (RCRA)). SYBR Safe stain meets the requirements of the U.S. Clean Water Act and the U.S. National Pollutant Discharge Elimination System (NPDES).	Keep in a dry, cool and well-ventilated place. Keep in properly labelled containers. Incompatible with: Strong acids. Strong oxidising agents	
GeneRuler 1 kb Plus DNA Ladder, ready-to-use		N/A	N/A	1 - Small	Choose an item.	The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique. Disposal of this product, its solutions or of any by-products, shall comply with the requirements of all applicable local,	Keep in a dry, cool and well-ventilated place. Keep in properly labelled container. Store at -20 °C.	

						regional or national/federal regulations.		

GHS symbols– SH (serious health hazard), AT (acute toxicity), H (health hazard), C (corrosive), Ex (explosive), F (flammable), O (oxidiser), Env (environment), CG (compressed gas). These should be obtained from chemical SDS documentation. See Appendix (hazard symbols).
Inhalation Group and Skin/Eyes Group- Hazard groups are classified as A/SA (least hazardous) to E/SE (most hazardous). See Appendix for hazard phrases associated with each group. Hazard phrases can be found on chemical SDS documentation.
Dustiness. Low (Pellet- does not break up), Medium (granular or crystalline), High (fine solid or light powder/dust)
Volatility. Low, medium, high, gas. Consider boiling point of liquid and operating temperature.
Disposal e.g. autoclaving of biohazard, SU chemical disposal
Primary containment: e.g. sealed flask, supplied vessel. **Storage:** e.g. secure chemical storage, fridge, freezer, general chemical storage

PROTOCOL RISK MANAGEMENT

Secondary Containment (of protocol): open bench/fume hood/special (highlight/state)
Working Practice – Good Laboratory Practice under local rules PLUS the following: (highlight/state)
Other risks & control measures e.g. pressure, temperature, electrical, mechanical, autoclave, field, boat.
Identify all specific control measures (highlight or delete) - latex/nitrile/heavy gloves; safety glasses, screens; full face mask; dust mask; protective shoes; spillage tray; ear-defenders; other (state) n.b. details for each chemical are in individual COSSH forms **Agarose**: Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses. Gloves material: Nitrile rubber Minimum layer thickness: 0.11 mm Break through time: 480 min Material tested:KCL 741 Dermatril® L. Respiratory protection required when dusts are generated, Filter type: Filter type P1.

TAE buffer: Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses. Gloves material: Nitrile rubber Minimum layer thickness: 0.11 mm Break through time: 480 min Material tested:KCL 741 Dermatril® L. Protective clothing. Respiratory protection required when vapours/aerosols are generated, Filter type: Filter type ABEK.

Nucleic acid stain (SYBR Safe): In case of insufficient ventilation wear respirators and components tested and approved under appropriate government standards. Wear suitable gloves Glove material: Compatible chemical-resistant gloves. Tight sealing safety goggles. Wear suitable protective clothing.

GeneRuler 1 kb Plus DNA Ladder, ready-to-use: In case of insufficient ventilation wear respirators and components tested and approved under appropriate government standards. Wear suitable gloves Glove material: Compatible chemical-resistant gloves. Tight sealing safety goggles. Wear suitable protective clothing.

Justification and controls for any work outside normal hours

Emergency procedures (e.g. first aid, spillage clearance; communication methods) n.b. full emergency plans for each chemical are detailed in individual COSSH forms

Agarose: Avoid inhalation of dusts. Evacuate the danger area, observe emergency procedures, consult an expert. Do not let product enter drains. Cover drains. Collect, bind and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up dry. Dispose of properly. Clean up affected area. Avoid generation of dusts.

TAE buffer: Do not breathe vapors, aerosols. Avoid substance contact. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, consult an expert. Do not let product enter drains. Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up with liquid-absorbent material (e.g. Chemizorb®). Dispose of properly. Clean up affected area.

Nucleic acid stain (SYBR Safe): Eliminate all ignition sources (no smoking, flares, sparks or flames in immediate area) Use personal protection equipment. Soak up with ine absorbent material.

GeneRuler 1 kb Plus DNA Ladder, ready-to-use: Ensure adequate ventilation. Always wear recommended Personal Protective Equipment. Use personal protection equipment. Soak up with inert absorbent material.

Supervision/training for worker (highlight) **N.B. All relevant training forms (e.g. for specific laboratories) should be attached**

None required Already trained Training required Supervised always

Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker..... Joe Jones

Name & counter-signature of supervisor.....	*Date.....*
Date of first reassessment	Frequency of reassessments

V. 2023

Risk Assessment for Teaching, Administration and Research Activities

Swansea University; FSE: Biosciences

Name Joe Jones **Signature ..... date** 25/03/2024

Supervisor* Eva Sonnenschein .....**Signature** [redacted] **date** 07/10/24

Activity title Agarose gel electrophoresis **Base location (room no.)** Wallace 123, 131A

University Activity Serial # (enter Employee No. or Student No.). [redacted]

Start date of activity (cannot predate signature dates) 25/04/2024

End date of activity (or 'on going') on going

Level of worker (choose from the list below) M.Res

UG, MSc, M.Res, M.Phil/PhD, RA/Postdoc, technician, administration, academic staff, visitor, other (state)

Ethics approval number 1 2024 9265 8148

Approval obtained for Biological Hazards and/or GMO Safety Assessment by SU? Yes/not applicable

Approval obtained for use of radioisotopes
Yes/not applicable

(If the answer is YES to any of the above, then other specific rules will apply -please seek advice)

Record of specialist training undertaken (see Training record document)

Course	Check appropiate

Summary of protocols used; protocol sheets to be appended and updated as necessary

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#	Assessment					#	Assessment				
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9						19					
10						20					

(Continue on another sheet if necessary)

Protocol Risk Assessment Form

(Expand or contract fields, or append additional sheets as required; insert NA if not applicable)

Protocol #HSEMK01	**Title: Agarose gel electrophoresis**
Associated Protocols #.....	**Location (**e.g. Boat, Field (give locations, Laboratory, Office, Facility). Identify here risks and control measures for work in this environment, additional to Local Rules: Wallace lab 131A

Description:

https://benchling.com/s/prt-hJJiAOFxFOrfma9u76tJ?m=slm-IYUP7ezygAbbWCyhRwAS

CHEMICAL RISK – Summary sheet

A copy of each Chemical COSSH form should be readily available in the lab for use (e.g. in an emergency)

Chemical Name (& Conc.) for chemicals to be used and generated	**GHS symbols** (SH, AT, H, C, Ex, F, O, Env, CG) All that are applicable.	**Skin/Eyes Group** (SA, SB, SC, SD, SE)	**Inhalation Group** (A,B,C,D,E)	**Quantity**	**In use dustiness or volatility**	**Disposal**	**Primary containment & storage**	**Other comments:** **In use factors affecting expo and special control measure** (e.g. <15 mins duration/ freque splash protection only/ hand immersion/ spraying) **Safety/ environmental hazar (H2XX/H4XX)**
Agarose		N/A	N/A	1 - Small	Low dustiness	Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. Notice Directive on waste 2008/98/EC.	Tightly closed. Dry. Storage class (TRGS 510): 11: Combustible Solids	

Tris-acetate-EDTA (TAE) buffer		SC	D	2 - Medium	Choose an item.	Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. Notice Directive on waste 2008/98/EC.	Keep containers tightly closed in a dry, cool and well-ventilated place. Storage class (TRGS 510): 12: Non-flammable liquids in non-flammable packages.	H226, H301, H310, H314, H31 H317, H318, H319, H330, H40 H410, H411
Nucleic acid stain (e.g. SYBR Safe)		N/A	N/A	1 - Small	Choose an item.	SYBR Safe DNA gel stain is not classified as hazardous waste under U.S. Federal regulations (Resource Conservation and Recovery Act (RCRA)). SYBR Safe stain meets the requirements of the U.S. Clean Water Act and the U.S. National Pollutant Discharge Elimination System (NPDES).	Keep in a dry, cool and well-ventilated place. Keep in properly labelled containers. Incompatible with: Strong acids. Strong oxidising agents	
GeneRuler 1 kb Plus DNA Ladder, ready-to-use		N/A	N/A	1 - Small	Choose an item.	The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique. Disposal of this product, its solutions or of any by-products, shall comply with the requirements of all applicable local,	Keep in a dry, cool and well-ventilated place. Keep in properly labelled container. Store at -20 °C.	

						regional or national/federal regulations.		

GHS symbols– SH (serious health hazard), AT (acute toxicity), H (health hazard), C (corrosive), Ex (explosive), F (flammable), O (oxidiser), Env (environment), CG (compressed gas). These should be obtained from chemical SDS documentation. See Appendix (hazard symbols).
Inhalation Group and Skin/Eyes Group- Hazard groups are classified as A/SA (least hazardous) to E/SE (most hazardous). See Appendix for hazard phrases associated with each group. Hazard phrases can be found on chemical SDS documentation.
Dustiness. Low (Pellet- does not break up), Medium (granular or crystalline), High (fine solid or light powder/dust)
Volatility. Low, medium, high, gas. Consider boiling point of liquid and operating temperature.
Disposal e.g. autoclaving of biohazard, SU chemical disposal
Primary containment: e.g. sealed flask, supplied vessel. **Storage:** e.g. secure chemical storage, fridge, freezer, general chemical storage

PROTOCOL RISK MANAGEMENT

Secondary Containment (of protocol): open bench/fume hood/special (highlight/state)
Working Practice – Good Laboratory Practice under local rules PLUS the following: (highlight/state)
Other risks & control measures e.g. pressure, temperature, electrical, mechanical, autoclave, field, boat.
Identify all specific control measures (highlight or delete) - latex/nitrile/heavy gloves; safety glasses, screens; full face mask; dust mask; protective shoes; spillage tray; ear-defenders; other (state) n.b. details for each chemical are in individual COSSH forms **Agarose**: Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses. Gloves material: Nitrile rubber Minimum layer thickness: 0.11 mm Break through time: 480 min Material tested:KCL 741 Dermatril® L. Respiratory protection required when dusts are generated, Filter type: Filter type P1.

TAE buffer: Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU). Safety glasses. Gloves material: Nitrile rubber Minimum layer thickness: 0.11 mm Break through time: 480 min Material tested:KCL 741 Dermatril® L. Protective clothing. Respiratory protection required when vapours/aerosols are generated, Filter type: Filter type ABEK.

Nucleic acid stain (SYBR Safe): In case of insufficient ventilation wear respirators and components tested and approved under appropriate government standards. Wear suitable gloves Glove material: Compatible chemical-resistant gloves. Tight sealing safety goggles. Wear suitable protective clothing.

GeneRuler 1 kb Plus DNA Ladder, ready-to-use: In case of insufficient ventilation wear respirators and components tested and approved under appropriate government standards. Wear suitable gloves Glove material: Compatible chemical-resistant gloves. Tight sealing safety goggles. Wear suitable protective clothing.

Justification and controls for any work outside normal hours

Emergency procedures (e.g. first aid, spillage clearance; communication methods) n.b. full emergency plans for each chemical are detailed in individual COSSH forms

Agarose: Avoid inhalation of dusts. Evacuate the danger area, observe emergency procedures, consult an expert. Do not let product enter drains. Cover drains. Collect, bind and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up dry. Dispose of properly. Clean up affected area. Avoid generation of dusts.

TAE buffer: Do not breathe vapors, aerosols. Avoid substance contact. Ensure adequate ventilation. Evacuate the danger area, observe emergency procedures, consult an expert. Do not let product enter drains. Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up with liquid-absorbent material (e.g. Chemizorb®). Dispose of properly. Clean up affected area.

Nucleic acid stain (SYBR Safe): Eliminate all ignition sources (no smoking, flares, sparks or flames in immediate area) Use personal protection equipment. Soak up with ine absorbent material.

GeneRuler 1 kb Plus DNA Ladder, ready-to-use: Ensure adequate ventilation. Always wear recommended Personal Protective Equipment. Use personal protection equipment. Soak up with inert absorbent material.

Supervision/training for worker (highlight) **N.B. All relevant training forms (e.g. for specific laboratories) should be attached**

None required Already trained Training required Supervised always

Declaration I declare that I have assessed the hazards and risks associated with my work and will take appropriate measures to decrease these risks, as far as possible eliminating them, and will monitor the effectiveness of these risk control measures.

Name & signature of worker..... Joe Jones

Name & counter-signature of supervisor.....	*Date.....*
Date of first reassessment	Frequency of reassessments