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Maintaining beneficial alga-associated bacterial communities under heat stress: Insights from controlled co-culture experiments using antibiotic-resistant bacterial strains

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One sentence summary: Inoculation with bacterial helper cultures that complement host metabolism increases the control over host-associated microbiome in the model brown alga *Ectocarpus*.

Abstract

Brown algae, like many eukaryotes, possess diverse microbial communities. *Ectocarpus* - a model brown alga- relies on these communities for essential processes, such as growth development. Controlled laboratory systems are needed for functional studies of these algal-bacterial interactions. We selected bacterial strains based on their metabolic networks to provide optimal completion of the algal metabolism, rendered them resistant to two antibiotics, and inoculate them to establish controlled co-cultures with *Ectocarpus* under continuous antibiotic treatment. We then monitored the stability of the resulting associations under control conditions and heat stress using 16S metabarcoding. Antibiotics strongly reduced bacterial diversity both in terms of taxonomy and predicted metabolic functions. In the inoculated sample, 63-69% of reads corresponded to the inoculated strains, and the communities remained stable during temperature stress. They also partially restored the predicted metabolic functions of the natural community. Overall, the development of antibiotic-resistant helper cultures offers a promising route to fully controlled laboratory experiments with algae and microbiota and thus represents an important step towards generating experimental evidence for specific host-microbe interactions in the systems studied. Further work will be required to achieve full control and progressively expand our repertoire of helper strains including those currently “unculturable”.

Introduction

As virtually all eukaryotes, brown algae are colonized by a diverse microbial community, together forming so-called holobionts (Dittami et al. 2021; Egan et al. 2013). The thallus of algae is more than a habitat for microbes; there are numerous dynamic inter-species interactions and metabolic exchanges between both compartments. Technological advancements in sequencing and metagenomics now permit the generation of valuable information about the composition of the host microbiome. Furthermore, computational models of metabolic networks can be used to predict complementarity sets between microbiome species and hosts (Belcour et al. 2020; Frioux et al. 2018; Levy et al. 2015).

Brown algae (Phaeophyceae) are important components of the flora on rocky shores in temperate and cold marine environments around the world, establishing critical ecosystem habitats such as kelp forests (Steneck et al. 2002). Many of these kelp forests, especially along the European coast of the North Sea, Eastern Australia, and California, are declining (Krumhansl et al. 2016; Smale 2020) - a phenomenon that is commonly attributed to anthropogenic impacts and global warming, though the precise causes are unknown (Provost et al. 2017).

Among brown algae, *Ectocarpus* (a small filamentous alga) has been established as a genetic and genomic model (Cock et al. 2010) and it is known to depend on the bacteria for proper development and for acclimation to low salinities (Dittami et al. 2016; Pedersen 1968; Tapia et al. 2016). According to a recent study, the co-culture of *Ectocarpus* with selected bacteria resulted in increased algal growth, and samples treated by different specific bacteria had diverging metabolite profiles and morphologies (Burgunter-Delamare et al. 2020).

Despite advances in our understanding of the diversity of bacterial symbionts and the prospective use of metabolic complementarity to test host-microbe interactions, there are still unanswered questions about symbiont acquisition and host-microbe balance in the face of abiotic stress. Furthermore, it is unresolved if and to what extent imbalances between algae and their associated microbiomes may play a role in ecologically important things like the decline of kelp forests. The establishment of controlled laboratory systems for brown algal-bacterial interaction would constitute a big advance in this field, enabling the mechanistical study of bacterial algal interactions in a changing environment, and the generation of testable hypotheses (van der Loos et al. 2019).

The aim of this study was to improve our control of the alga-associated microbiome. Given the strong interdependencies between the algal hosts and their associated microbiome, this challenge was addressed by providing the alga with a defined set of antibiotic-resistant ‘helper bacteria’. These bacteria were selected from a collection of host-derived bacteria, based on their predicted metabolism, to be able to produce the maximum number of compounds that are missing for the predicted metabolism of the host (Dittami et al. 2014). In addition, the algae and bacteria were kept under continuous antibiotic treatment to reduce the proliferation of other non-target strains that might colonize the niche opened up by the antibiotic treatment. The suitability of this approach was tested in the algal host model *Ectocarpus*, and the stability of the microbiome was assessed under both control conditions and under heat stress.

98 **Materials &Methods**

99 **Beneficial bacterial consortium selection**

100 *In silico* selection of beneficial bacteria was performed in a previous study (Karimi et al.
101 2021a). Briefly, 81 bacterial genomes isolated from *Ectocarpus* (Karimi et al. 2019) were
102 annotated using Prokka v 1.13 (Seemann 2014). Subsequently, bacterial metabolic networks
103 were constructed using Pathway Tools version 23 and the mpwt wrapper implemented in the
104 AuCoMe pipeline (Belcour et al. 2020). The bacterial metabolic networks and the available
105 host metabolic network (Dittami et al. 2020) were submitted to MiSCoTo as SMBL files
106 (Frioux et al. 2018). The latter tool was first used to determine the scope of all algal
107 compounds that, according to the metabolic models, and without limiting metabolite
108 exchange, became producible via exchanges between the host and all 81 bacterial genomes
109 together: a total of 506 compounds listed in Supplementary Table S8 of Karimi et al. (2021a).
110 The same tool was then used to determine the minimal bacterial consortia that enabled the
111 production of these compounds. A total of 18 minimal communities was predicted each
112 comprising 10 bacterial strains (listed in Supplementary Table S1), and the union of all
113 minimal consortia contained 15 bacteria. All of these 15 bacteria and their taxonomic
114 affiliations are listed in Supplementary Table S1, and we attempted to generate antibiotic-
115 resistant strains for each one of them. The host and bacterial networks have also been
116 deposited in the Zenodo repository (Karimi et al. 2021b).

117 **Antibiotic sensitivity testing on algae**

118 Filaments of the brown alga *Ectocarpus subulatus* strain Bft15b were cultured in 10-liter
119 Nalgene™ Round Polycarbonate flasks with Provasoli-enriched autoclaved seawater for 15
120 days. Temperature was maintained constant at 16°C, and constant airflow and a light-dark
121 cycle of 12:12h were applied in controlled culture chambers. The algal filaments were then
122 placed in 90mm sterilized Petri dishes filled with Provasoli-enriched artificial seawater
123 containing the following components: NaCl 450mM, MgSO₄(7H₂O) 16mM, MgCl₂(6H₂O)
124 30 mM, KCl 10mM, CaCl₂ 9mM adjusted to pH 7.8 with 10mM Tris. Culture conditions
125 remained identical, except for the absence of bubbling. To obtain axenic algae, the following
126 antibiotic treatment was selected based on the work of Cottrell and Suttle (1993) and
127 preliminary experiments in our laboratory: three-day exposure to a combination of
128 Chloramphenicol (CHL 9.6 µg/ml) and Neomycin (NEO 24.8µg/ml). This treatment

efficiently reduced associated bacteria but caused no visible damage (discoloration, rupture) to the algal cells.

Antibiotic sensitivity tests for bacteria and the generation of antibiotic-resistant strains

Disk diffusion tests were carried out to determine the susceptibility of the selected beneficial bacteria to chloramphenicol (CHL) and neomycin (NEO). Freshly cultured bacterial isolates were spread evenly on the R2A agar plates prepared with natural seawater, and grown in the presence of 30 µg CHL and 30 UI NEO antibiotic discs (Bio-Rad, Nanterre, France (Matuschek et al. 2014) for up to one week at 18°C. Next, we attempted to generate antibiotic resistance in the selected strains. To this means, bacteria were grown in 25 ml of liquid R2A medium in Corning® cell culture flasks at 25°C with agitation (50 rpm) supplemented with 0.3 µg/ml of CHL. Once bacterial growth was visible, 1 ml of culture was transferred to a new flask with a higher concentration and this process continued. Based on the “adaptation on demand” hypothesis (Johnsen et al. 2021), the increase in antibiotic concentration leads bacterial population to evolve resistance to the target antibiotic, until growth was observed at the target concentration of CHL (Supplementary Table S2). At this point, the disk diffusion test was repeated to check the obtained resistance (Table 1). Then, the same procedure was carried out starting with 3.93 µg/ml Neomycin (NEO) for the CHL-resistant strains. Finally, the identity of bacterial strains resistant to both antibiotics was confirmed by sequencing the 16S rRNA gene with the universal primers 27F and 1492R. After reaching the desired antibiotic resistance, the strains were preserved in 20% (vol/vol) glycerol at -80°C.

Co-cultures of algae and bacteria

For our co-culture experiments, we constructed a set that included all seven bacteria for which resistant strains were obtained. This set corresponds to the predicted minimal community 11 in Supplementary Table S1, but lacks *Frigoribacterium* sp. 9N, *Bacillus* sp. 9J, and *Novosphingobium* sp. 9U. These bacteria were grown in liquid R2A media with both antibiotics at their final target concentration. Once bacterial growth was visible with the naked eye (approximately 5 days), the optical density (OD) of the cultures was determined using Genova plus analyzer UV/Vis life science® spectrophotometer at 600 nm. All cultures were diluted to an OD of 0.1 for the inoculation of the algal cultures. For the co-culture set-up, algal filaments were dispersed in Petri plates (125 ml cultures), prepared with the two liquid antibiotics as described above, and inoculated with 500 µl of each resistant cultured

bacterium (OD = 0.1). These cultures, along with a set of controls, were then used in the stress response experiments described below.

Temperature stress experiment

The design of the stress response experiments, as well as the controls included are described in Fig. 1. Briefly, at the start of the experiment (t_0), algal-bacterial co-cultures were inoculated as described above (INOCUL), and in addition, a non-antibiotic treated positive control (NATUR), and an antibiotic-treated non-inoculated alga as a negative control (ATB) were included. For all three of these conditions, we prepared 8 replicate Petri dishes with 4 filaments in each (a total of 96 algal filaments). After the acclimation period of 10 days at 16°C (t_{10}) in a culture chamber at 16°C, 4 of the 8 replicates for each treatment were placed in a separate but otherwise identical culture chamber and subjected to a temperature increase of 2°C every two days for 6 days (t_{12} , t_{14} , t_{16}). The other 4 replicates were maintained at 16°C. At each of these time points, algal samples (one filament per Petri dish) were collected in Eppendorf tubes and immediately frozen in liquid nitrogen, and later at -80 °C for downstream analyses. Furthermore, at all steps of the experiment (Fig. 1), the general algal morphology was observed. Finally, at t_{16} , aliquots (15 ml) of the culture media were taken, centrifuged at 10,000 g for 30 min to pellet the bacterial cells present, and the pellets were stored at -80 °C for downstream analysis.

Metabarcoding profiling of co-culture experiments

A metabarcoding approach was implemented to investigate the composition of the bacterial community after the co-culture experiments and temperature disturbances. For each culture condition, frozen algal tissue was freeze-dried and ground (TissueLyserII; Qiagen, Hilden, Germany; 2×60 s, 30 Hz). The powder obtained was then used for DNA extraction (the Macherey-Nagel NucleoSpin 96 Plant II Kit). The DNA extraction for bacterial pellets collected from seawater media was performed using the Wizard® Genomic DNA Purification Kit (Promega, Madison, USA). DNA integrity was verified with gel electrophoresis. Two blank controls were included as negative controls in addition to the samples. Libraries were prepared according to the standard Illumina protocol for metabarcoding MiSeq technology targeting the V3–V4 region (Illumina, 2017). Briefly, the amplicon PCR was performed using NOCHL primer sets (Thomas et al. 2020). The PCR products were then purified using AMPure® XP beads (Agencourt Bioscience, Beverly, MA, USA) followed by index PCR in

KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, USA) to attach dual indices and Illumina sequencing adapters to each sample using the Nextera XT Index Kit (Epicentre, Madison, WI, USA). A second PCR clean-up was then carried out as described above. The resulting libraries were checked using a bioanalyzer (DNS 1000) to verify fragment size and a Qubit® fluorimeter (Life Technologies, Paisley, UK) for DNA quantification. An equimolar pool of all libraries was subjected to paired-end (2×300 bp) sequencing on a single flow cell of an Illumina MiSeq sequencer using the MiSeq v3 kit (Illumina, San Diego, CA, USA) on Platform GENOMER, FR2424, Roscoff.

Sequence analysis

The resulting raw sequences (10,508,975 read pairs) were processed using DADA2 within the R (v4.0.0) statistical computing environment (Callahan et al. 2016). Reads underwent quality filtering as error rates were calculated and removed from the dereplicated reads. An initial Amplicon Sequence Variant (ASV) table was constructed, and later chimeras were identified using the removeBimeraDenovo function. Finally, taxonomy was assigned using DADA2's native RDP Bayesian classifier against the Silva database version 138 (McLaren 2020). Non-bacterial sequences as well as all ASVs with less than ten reads or which were more abundant in the negative control compared to the samples were removed. No rarefaction was conducted but the 'calc_obs_props' function of the 'metacoder' package version 0.3.4 was used to calculate proportions and the abundance of taxa was computed using the 'calc_taxon_abund' function. For diversity analysis, the 'vegan' package was employed using the 'diversity' function. PICRUSt2 (v2.4.1) was used to predict functional traits of bacteria based on their marker gene sequences in each treatment with default settings (Douglas et al. 2020).

Statistical analyses

All statistical analyses and graphs were performed within the computing environment R (v4.0.0; R Core Development Team, 2005) using the packages phyloseq (McMurdie and Holmes 2013), phylosmith (Smith 2019), vegan (Oksanen et al. 2020), ggplot2 (Valero-Mora 2010), metacoder (Foster et al. 2017), microbiome (Lahti et al. 2017). Statistical tests were considered significant at $\alpha = 0.05$. Differences in community composition (β -diversity) were computed using Bray–Curtis dissimilarity matrices and tested via permutational multivariate

analysis of variance (PERMANOVA) and the statistical analysis of the predicted pathway data was performed using STAMP version 2.1.3(Parks et al. 2014).

Results

Antibiotic resistance and sufficient growth obtained for 7 out of 15 bacteria.

To set up a controlled co-culture experiment, we continuously applied antibiotics. Therefore, we needed to generate antibiotic-resistant strains of the bacteria we had selected based on metabolic complementarity. All 15 of the initially bacteria developed resistance to CHL and subsequently to neomycin (NEO) (Supplementary Table S2). For 10 out of these 15 strains the resistance to both antibiotics (Supplementary Figure S1) was durable and 7 strains out of 10 exhibited growth rates that were sufficient to prepare co-culture experiments within a timescale of 5 days (Table1)

Metabarcoding survey of algal samples

In total, 71 samples from three algal treatment conditions were considered for the core analysis of the study: bacterial inoculation with ATB treatment (n = 24, labeled as INOCUL); no inoculation and no ATB treatment (n = 23, here known as NATUR), and no bacterial inoculation with ATB treatment (n = 24, labeled as ATB). One sample replicate from the ATB treatment was removed alongside the negative controls because insufficient reads were obtained. Of 10 million raw reads were made up 1 005 455 unique sequences belonging to 1 933 different ASVs. The ASVs and taxonomy tables are provided in Supplementary Table S3.

Six inoculated resistant bacteria out of seven persisted in cultures.

Six inoculated bacteria out of seven were detected in metabarcoding data (Fig. 2). Only *Rhizobium* had no reads although this strain had developed resistance to both antibiotics in a timely manner. Overall, the inoculated strains were detected specifically in the conditions they were inoculated in, except for *Maribacter* which was present in all cultures (Fig. 2). In the samples that underwent the INOCUL treatment, reads corresponding inoculated bacteria,

amounted to 66% of total reads (69% in control conditions and 63% in stress conditions, Supplementary Figure S2).

Antibiotic treatments reduce bacterial diversity and modify community structure.

The Shannon diversity indices per sample ranged from 3.33 to 4.77 (Fig. 3, See Supplementary Table S4 for additional diversity indices). They differed significantly between ATB-treated, natural, and ATB-treated inoculated samples (two-way ANOVA, $p < 0.001$), but differences between temperature-stressed and control samples were not significant in the same analysis ($p = 0.28$; Fig. 3). Generally, the communities from samples inoculated with the selected bacteria (INOCUL) exhibited lower phylogenetic diversity than control (NATUR) and higher than the antibiotic treated (ABT).

Non-metric Multidimensional Scaling (nMDS) visualization of β -diversity using the Bray–Curtis dissimilarity index revealed a clear separation of co-culture conditions (Fig. 4; PERMANOVA, Bray–Curtis method, 999 permutations; $R^2 = 0.75$, $p < 0.001$). However, no clear differences between stress- and control samples were observed for the overall community composition. PERMANOVA analyses carried out separately for ATB, INOCL, and NATUR samples after inculcation (t_{10} - t_{16}) also revealed that the community composition changed slightly but significantly in response to stress for the ATB and INOCL treatments ($p=0.013$ and $p=0.041$, respectively) but not for the NATUR treatment.

Taxonomic composition of bacterial communities

The taxonomic composition of bacterial communities in ATB, INOCUL, and NATUR cultures is illustrated in Fig. 5A, where only the non-inoculated strains are considered (inoculated strains are shown in Fig. 2). In the natural samples, we observed a complex community dominated by sequences corresponding *Flavobacteriales*, *Caulobacterales*, *Oceanospirillales*, and *Rhodobacterales*. ATB treated samples including the INOCUL samples (without the inoculated bacteria), were dominated by sequences belonging to *Flavobacteriales* and more specifically the genus *Winogradskyella*, and only 5-10% of reads corresponded to other groups.

The pairwise comparisons in Fig. 5B display the relative abundance of each observed taxonomic group in stress versus control samples. Here, nodes represent taxonomic levels, and each branch represents the phylogenetic relationship between those entities. ASV richness is represented by node size and the relative abundance of reads corresponding to this

node by color. For the natural microbiota, this heat tree shows few taxonomic differences in the composition in response to stress, notably an increase in the proportion *Cytophagales* including *Fabibacter*, and a decrease in *Pseudomonadales*. The ATB in INOCL treatment exhibit a few common responses to stress, such as the increase in the part of *Bacillales* in response to stress, and a decrease in *Gammaproteobacteria* and *Rhodobacteriaceae*. Specifically, in the INOCL treatment, we also observed a relative increase in *Micrococcales* and *Rhizobiaceae* in response to stress.

Taxonomy-based functional profiling of bacterial communities in control and stressed samples

In addition to generating the taxonomic profiles, we used Picrust2 to functional profiles based on the metabarcoding data. During this process, 15 ASVs with [Nearest Sequenced Taxon Index](#) (NSTI) values of more than 2 were eliminated, along with six ASVs with poor alignment (Barbera et al. 2018; Czech et al. 2020; Louca and Doebeli 2017; Mirarab et al. 2012). The average of NSTI values for our samples (Supplementary Table S5) was 0.047 ± 0.007 (mean + SD), with slightly higher NSTIs (0.053 ± 0.003) for the NATUR samples than the INOCUL samples (0.044 ± 0.009), [indicating the availability of close reference genomes in the PICRUST2 database](#). Principal component analyses of these profiles revealed that ATB, INOCL, and NATUR samples were clearly associated with distinct functional profiles (Fig. 6A). Using a Kruskal-Wallis test and a Benjamini Hochberg correction, we found that 314 out of 349 predicted metabolic pathways differed significantly ($p_{adj} < 0.05$ in at least one of these conditions; Supplementary Table S5). Interestingly, from a functional point of view, INOCL samples were closer to the NATUR samples than the ATB samples (Fig. 6B). However, none of the predicted metabolic pathways differed significantly in response to stress when analyzing the ATB, INOCL, and NATUR groups separately.

Discussion

The effect of antibiotic treatments on algal cultures

Protocols to obtain truly axenic cultures using antibiotic treatments are available for several microalgal species (e.g., Cirri et al. 2018; von Dassow et al. 2009). For macroalgae axenic cultures are, to the best of our knowledge, available only for *Ulva mutabilis* (Califano and Wichard 2018) and even these do not exhibit normal growth and morphology unless complemented with bacterial morphogens (Spoerner et al. 2012). In most cases, e.g. the filamentous brown alga *Ectocarpus* (Burgunter-Delamare et al. 2020) but also some microalgae (Geng et al. 2016) antibiotic treatments reduced bacterial abundance and led to a shift in bacterial community composition. The example of *Ectoacrpus subulatus* shows that this reduction may be temporary and that the relative bacterial abundance, as estimated by the number of bacterial vs. host reads in metatranscriptomic experiments, may even increase after recovery from strong antibiotic treatments (KleinJan et al. 2023). In our study, the comparison of the NATUR and the ATB treatments confirms a change in the bacterial community. In our case, the community shifted from a diverse assembly of Proteobacteria to a community dominated by Bacterioidetes and notably the genus *Winogradskyella* (Flavobacteria). This is similar to previous results by Burgunter-Delamare et al. (2020) where another, yet undescribed genus of Flavobacteria became dominant after antibiotic treatments. *Winogradskyella* is frequently associated with marine mammals and algae, and uncultivable strains of the genus have been proposed to have specialized in the utilization of the brown algal polysaccharide laminarian while reducing other aspects of their metabolism (Alejandre-Colomo et al. 2021). Our current working hypothesis is that if there is an open niche created by the antibiotic treatment it will be filled by resistant or previously dormant bacteria that are well-adapted to live on macroalgae. We may furthermore hypothesize that the newly established bacteria may, at least in some cases, be less beneficial to the algal host, and in any case, we have no control over the communities that will establish themselves.

Inoculation stabilized bacterial communities and partially restores metabolic functions

In this study, we attempted to fill the empty niche created by an antibiotic treatment by inoculation with a controlled and beneficial bacterial community. At the same time, we gave this target community a selective advantage by maintaining an antibiotic treatment. Our data show that this approach worked partially – approximately 63-67% of bacterial reads obtained corresponded to the inoculated bacteria, and only about 31%-37% to others. Although this is still a considerable amount of non-target bacteria, in a comparable study

without continuous antibiotic treatment, on average only 11% of the final reads corresponded to the inoculated bacteria (see Burgunter-Delamare et al. (2020), notably Table 4 therein). Furthermore, our Picrust2 analyses underline that the obtained bacterial communities are functionally more similar to the “natural” laboratory communities than the communities after only ATB treatment (Fig. 6). This indicates that the inoculation with selected bacteria was at least to some extent able to restore the functions naturally present in the microbiome.

Bacterial communities had little influence on host temperature stress response or vice versa

In *Ectocarpus*, bacterial communities have also been shown to have a significant impact on the holobiont salinity response and vice versa (Dittami et al. 2016; KleinJan et al. 2023). We, thus, wanted to test if our approach of using antibiotics and antibiotic-resistant cultures could help stabilize beneficial bacterial communities, and by extension increase holobiont stress tolerance. Unlike in previous experiments with strong changes in salinity, our results on temperature stress exhibited only weak changes in the bacterial community in both the INOCL and in the control (ATB, NATUR), thus making it impossible to conclude on stabilizing effect. Please note, that the absence of an impact of stress on the bacterial communities was observed, despite the strong intensity of the temperature stress. Indeed, at the end of the experiments, we had reached the limit of the thermal tolerance range of our strains: an additional increase in temperature by two degrees led to rapid algal bleaching and death regardless of the treatment (ATB, INOCL, NATUR; data not shown).

One possible explanation for the discrepancy between the previous salinity tolerance experiments may be the different effects the stressor had on the host or the microbiome. *Ectocarpus subulatus* is a euryhaline species frequently found in estuaries. It is, as such, well-adapted to strong fluctuations in salinity (Peters et al. 2015). The associated microbiome of the strain used in Dittami et al (2016), however, might have impoverished and evolved over the 20 years of cultivation in the laboratory, since its original isolation in 1995 (West and Kraft 1996) and marine-freshwater transitions in general, are considered rare among microbes (Logares et al. 2009). Regarding temperature changes, the algal hosts, on the other hand, generally have narrow temperature tolerance windows, and maximum summer temperatures are one of the factors limiting their distribution (Lüning 1984) Regarding bacteria, even bacteria considered psychrophilic usually grow well at 20°C (Morita 1975), and most (mesophilic) bacteria tolerate up to 45°C (Willey et al. 2008) Although this is presently only

a hypothesis, is important to keep in mind the possibility of differences in the stress response of the host and the different symbiotic partners. Depending on which component is affected different approaches may be appropriate when attempting to manipulate the system.

Bacterial manipulation with antibiotic-resistant bacteria – a promising but low throughput strategy

While our experiments show a clear increase in the control over the *Ectocarpus*-associated bacterial communities, we did not reach complete control over the alga-associated microbiome, and several aspects of these experiments could be improved. First, as illustrated by the metabolic distance between the INOCL and the NATUR communities, we are not yet completely substituting the natural communities in terms of metabolic capacities; This may be related in part to the fact that we were able to co-culture only six of the initially selected 16 bacterial strains. At the same time, we are also still limited to cultivable isolates. Increasing our efforts on cultivating the “unculturable” (Zengler et al. 2002) would therefore also help cover a larger portion of the bacterial metabolism in co-culture experiments. In parallel, additional effort, and the use of more or different antibiotics may help increase the control over the remaining alga-associated microbiota. Here, however, a balance needs to be struck, as antibiotic resistance for the bacteria also comes with a cost, and evolving resistances may further alter bacterial physiology (Händel et al. 2013) thus potentially impacting interactions with the host. Another limitation of our approach is the time it takes to prepare these cultures and co-culture experiments. Experiments with one or two bacterial strains of interest are easily feasible, but they are difficult to upscale because each bacterium reacts differently to antibiotics, and sometimes resistances are not stable making repeated treatments necessary. It will thus be more difficult to envision this approach for medium- or high-throughput screening of bacteria in a co-culture context, especially if complex communities are to be tested.

Despite these limitations, we believe that the development of a collection of antibiotic-resistant helper cultures for our algae constitutes one promising avenue towards fully controlled laboratory experiments with algae for which there are currently no working axenization protocols. If these cultures can fulfill the metabolic functions of the natural microbiome, their addition will thus enable increased experimental control over the microbiome during experiments, enhancing the reproducibility of microbiome-related work.

In the long run we could then progressively aim towards replacing the microbes by the metabolites or metabolic intermediates they provide.

Data availability

The Illumina sequence data generated in this study (Raw MiSeq data) have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB57188.

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Conflict of interest

The authors declare that they have no conflict of interest.

Figures and Tables

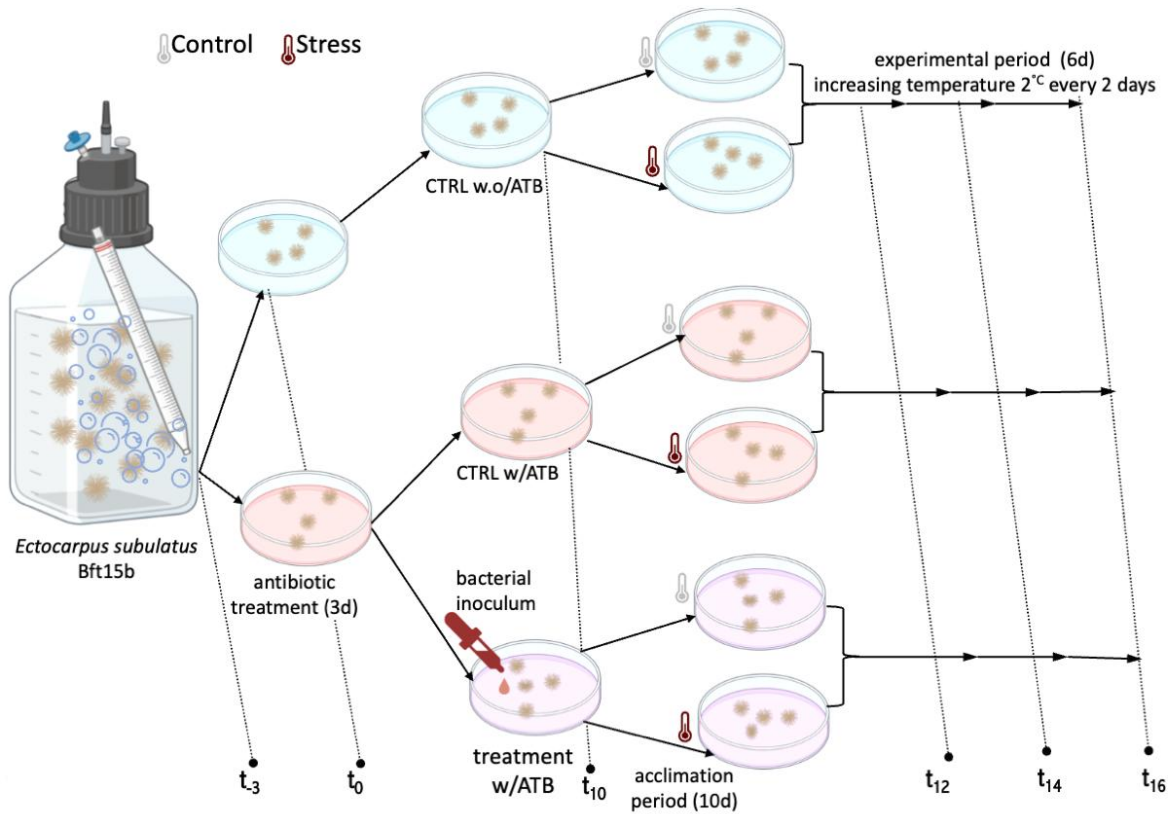


Fig. 1. A schematic illustration of the sampling design and experimental procedure. *Ectocarpus subulatus* was cultivated from stock populations of strain Bft15b. In the climate chamber (16°C) algae were grown for 7 weeks and divided into two series in petri dishes. One of the series was treated for three days with a mixture of two antibiotics (CHL & NEO), after which this group were divided into two series and one series received inoculum of 7 different bacteria. All samples stayed at the same temperature for 10 days. Later, replicates of each condition were moved to higher temperatures where the temperature was increased every 2 days in 2°C. Microbiota samples were taken directly after their separation (t_{-3}), before inoculation (t_0), after acclimation (t_{10}), and after 4 and 6 days (t_{14} and t_{16}). Parts of the figure were created with BioRender.com.

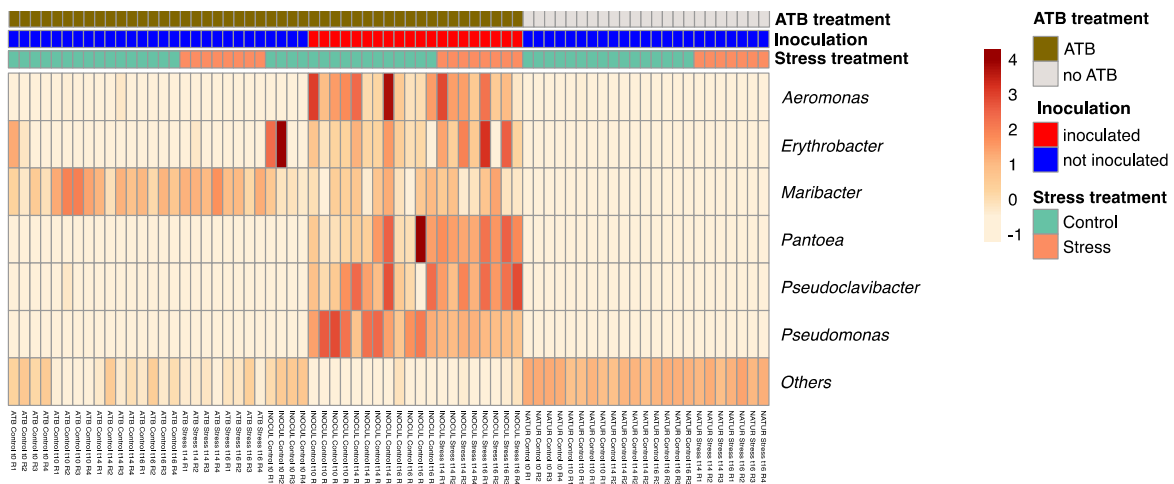


Fig. 2. Heatmap of relative read abundances corresponding to inoculated bacteria vs. other bacteria. Rows are centered; unit variance scaling is applied to rows.

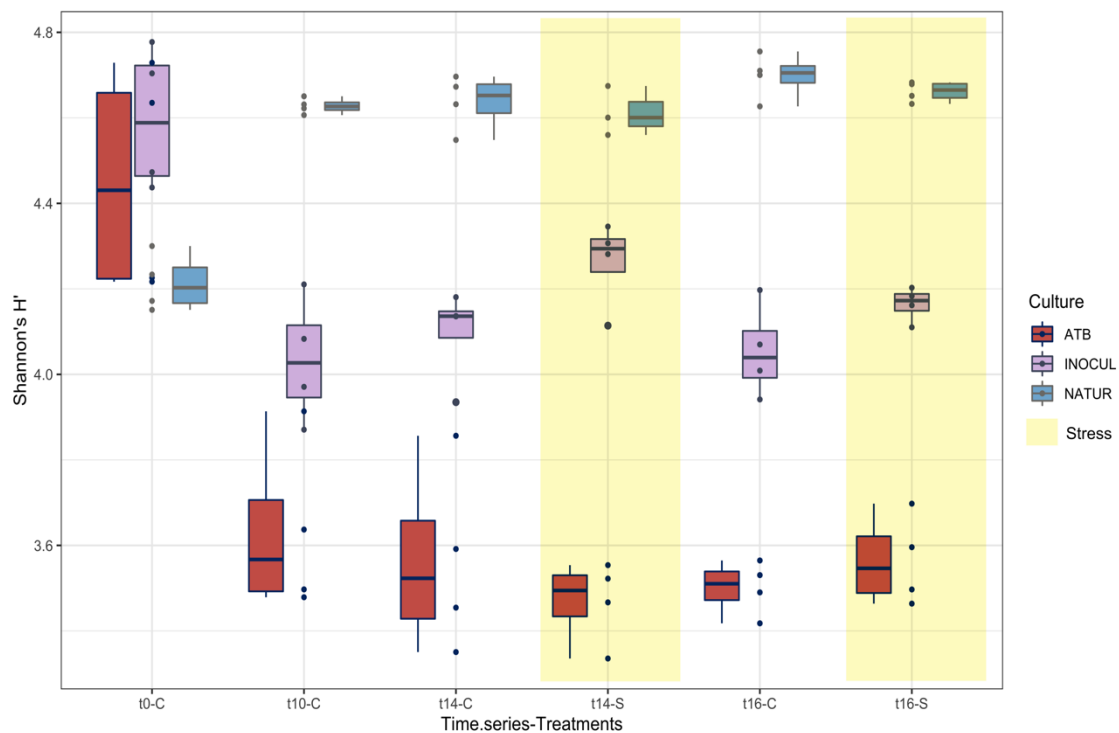


Fig. 3. Bacterial Alpha diversity. The dot-plot figures show the Shannon diversity of the bacterial communities in the three culture treatment groups, ATB, INOCUL, and NATUR, and at different time points in either control (C) or stress conditions (S). Calculations are based on the relative abundance of observed ASVs.

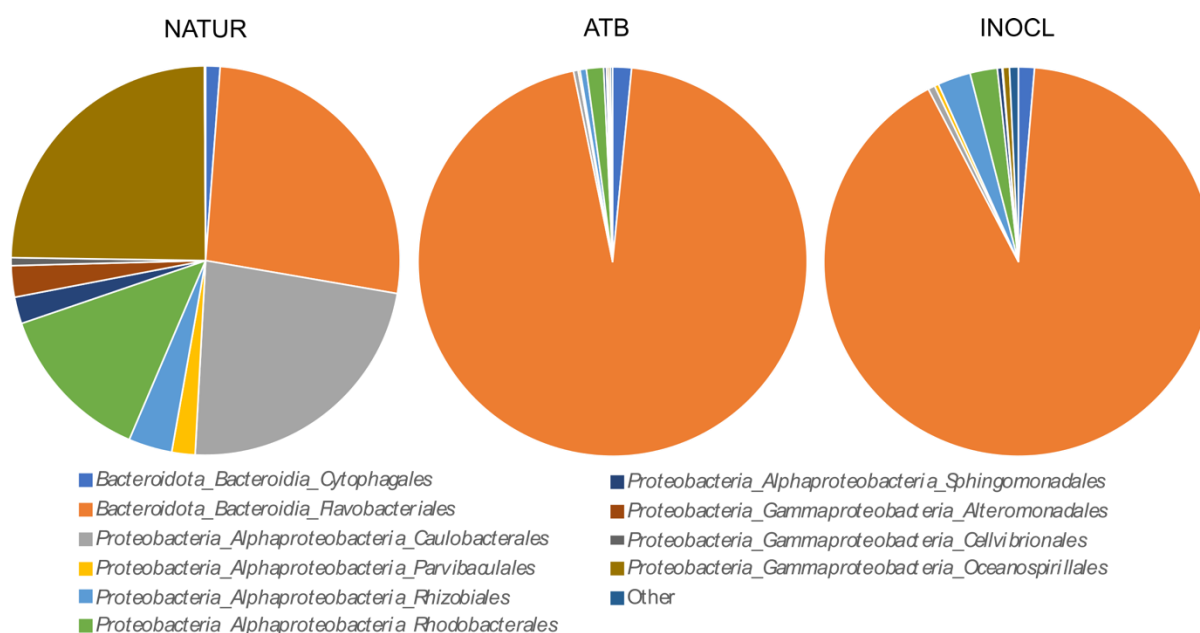


Fig. 4. Bacterial community composition varies based on algae culture conditions. NMDS visualization of beta diversity of the bacterial communities in culture conditions and in time series in stress and control treatments (C for control and S for stressed), based on Bray–Curtis dissimilarity matrices. Each point is an individual replicate. Based on PERMANOVA testing, temperature changes had no significant effects; PERMANOVA results for the comparison of ATB, INOCL, and NATUR are displayed in the figure.

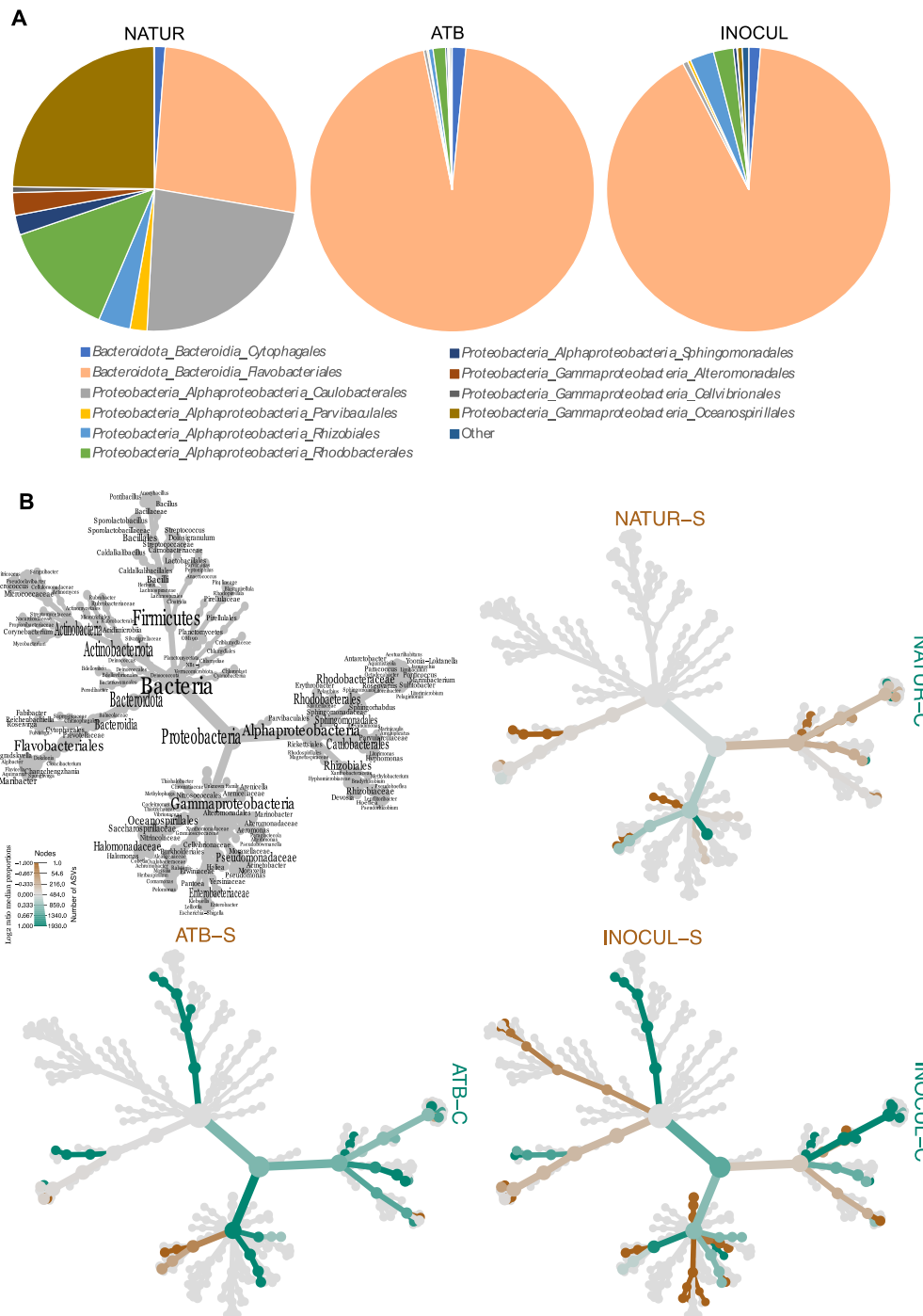


Fig. 5. A) Bacterial community in three culture conditions at the order level. Taxon with abundances more than 0.1% for all three conditions (the remaining were grouped as “others”). Only non-inoculated taxa were considered for this filter (please refer to Figure 2 for the inoculated taxa), and only samples after T10 (= time of inoculation), to keep the three conditions comparable. B) Differential heat tree matrix depicting the change in relative read abundance for the different taxa over different conditions and cultures. The number of taxa identified at each taxonomic level is represented by the size of the individual nodes in the gray cladogram. The smaller cladograms show pairwise comparisons between each culture condition: a brown node indicates that the taxon is more abundant in culture condition labeled in brown than in the one labeled in green and vice versa (C stands for Control (16 oC) and S stands for stressed (20 and 22 oC)).

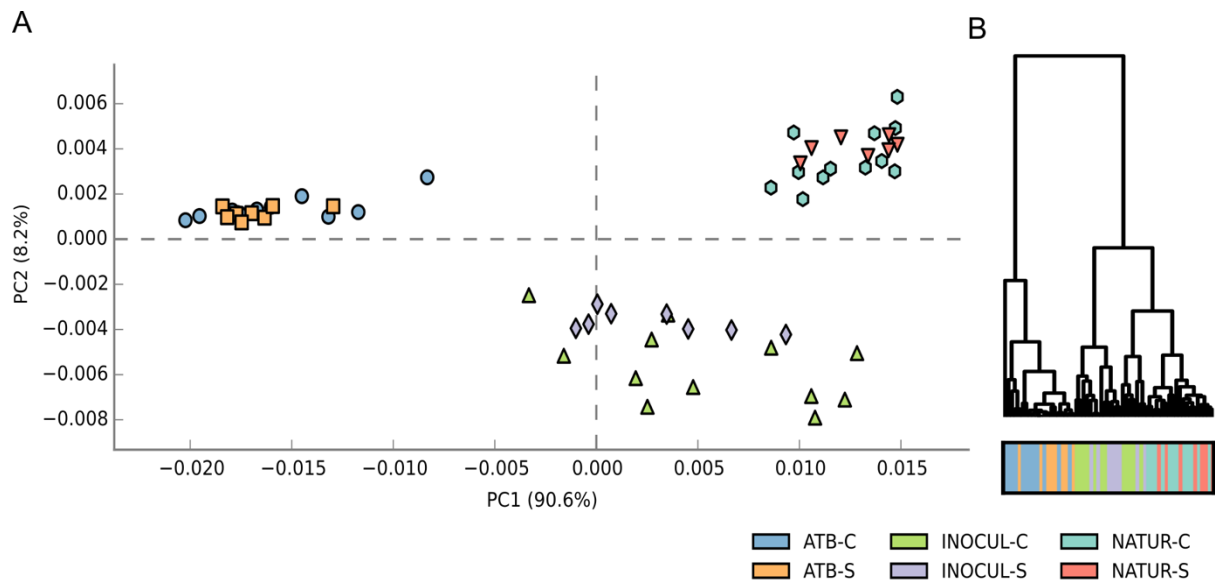
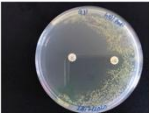
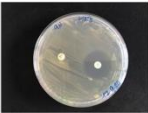


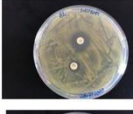



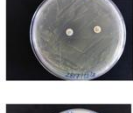
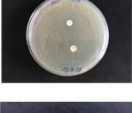

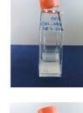
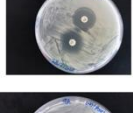
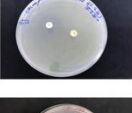


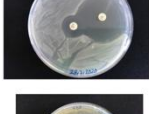
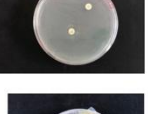


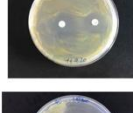
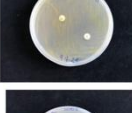
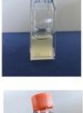

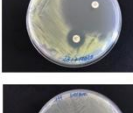
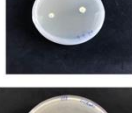

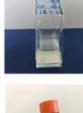
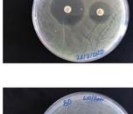
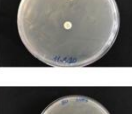

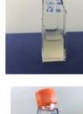
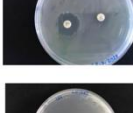
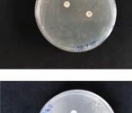


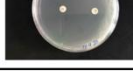





Fig. 6. A) Principal Component Analysis of predicted Metacyc pathways (Picrust2). B) Corresponding average neighbor UPGMA clustering.

Table 1. The result of disk diffusion test for susceptibility of selected bacteria to CHL & NEO antibiotics before and after resistance. Optical density at 600 nm (OD600) was measured after 5 days of growth in liquid media containing both CHL and NEO antibiotics.

	Zone of inhibition (mm)				Growth in liquid media (OD600 after 5 days)
	Before gaining antibiotic-resistance		After gaining antibiotic-resistance		
Selected bacteria consortia	CHL	NEO	CHL	NEO	CHL & NEO
<i>Frigoribacterium</i> sp. 9N	61	16	26	0	0.000
<i>Pseudoclavibacter</i> sp. 8L	18	10	0	0	1.186
<i>Maribacter</i> sp. 151	15	0	0	0	0.184
<i>Bacillus</i> sp. 9J	19	22	0	0	0.000
<i>Aeromonas</i> sp. 9A	43	18	0	0	0.671
<i>Erythrobacter</i> sp. EC-HK427	23	21	0	0	0.128
<i>Novosphingobium</i> sp. 9U	51	18	0	0	0.001
<i>Pantoea</i> sp. 111	33	23	0	0	0.432
<i>Pseudomonas</i> sp. 8O	0	22	0	0	0.301
<i>Rhizobium</i> sp. EC-SD404	56	17	0	0	0.145

Selected bacteria consortia	Before ATB resistant	After ATB resistant	Liquid media Inoculation Before ATB resistant	Liquid media Inoculation After ATB resistant
<i>Frigoribacterium</i> sp. 9N				
<i>Pseudoclavibacter</i> sp. 8L				
<i>Maribacter</i> sp. 151				
<i>Bacillus</i> sp. 9J				
<i>Aeromonas</i> sp. 9A				
<i>Erythrobacter</i> sp. EC-HK427				
<i>Novosphingobium</i> sp. 9U				
<i>Pantoea</i> sp. 111				
<i>Pseudomonas</i> sp. 8O				
<i>Rhizobium</i> sp. EC-SD404				

479
480 **Figure S1.** The appearance of normal and antibiotic resistant strains on R2A agar and liquid
481 R2A medium. The antibiotic discs correspond to Neomycine and Chloraphenicolcol,
482 respectively. The liquid ABT treatment corresponded to a mixture of Chloramphenicol (9.6
483 µg/ml) and Neomycin (24.8µg/ml). Photos were taken 5 days after inoculation

484
485 **Table S1.** Overview of mininal bacteria consortia prediacted by MiSCoTo and of Antibiotic
486 resistance survey.
487

Strain	ID	Phylum	Class	order	Family	Genus	Genome Completeness	ATB resistance obtained	Part of predicted community number																		Inoculated at OD = 0.1, 500ul
									1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
Aeromicrobium sp. 9AM	9AM	Actinobacteri	Actinobacteri	Actinobacteri	Actinomyceti	Aeromicrobi	99.7409	TRUE																			
Frigoribacterium sp. 9N	9N	Actinobacteri	Actinobacteri	Actinobacteri	Actinomyceti	Frigoribacter	98.4848	FALSE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Micrococcus sp. 116	116	Actinobacteri	Actinobacteri	Micrococcale	Micrococcae	Micrococcus	98.6386	FALSE	X	X	X	X	X	X							X	X	X	X	X	X	
Pseudoclavibacter sp. 8L	8L	Actinobacteri	Actinobacteri	Actinomyceti	Actinomyceti	Pseudoclavi	98.2456	TRUE						X	X	X	X	X	X	X							Inocul.
Flavobacterium sp. 9AF	9AF	Bacteroidetes	Flavobacterii	Flavobacteria	Flavobacteria	Flavobacteria	98.9399	TRUE	X	X	X				X						X		X				
Flavobacterium sp. 9R	9R	Bacteroidetes	Flavobacterii	Flavobacteria	Flavobacteria	Flavobacter	99.64	FALSE		X	X					X		X					X		X		
Maribacter sp. 151	151	Bacteroidetes	Flavobacterii	Flavobacteria	Flavobacteria	Maribacter	99.67	TRUE				X							X	X					X	X	Inocul.
Bacillus sp. 9J	9J	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	99.58	FALSE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Aeromonas sp. 8C	8C	Proteobacteri	Gammaprote	Aeromonada	Aeromonada	Aeromonas	100	FALSE				X	X	X			X	X		X			X	X	X	X	
Aeromonas sp. 9A	9A	Proteobacteri	Gammaprote	Aeromonada	Aeromonada	Aeromonas	100	TRUE		X	X		X	X	X	X				X		X	X	X	X	X	Inocul.
Erythrobacter sp. EC-HK427	430	Proteobacteri	Alphaproteob	Sphingomoni	Erythrobacter	Erythrobacter	99.1354	TRUE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Inocul.
Novosphingobium sp. 9U	9U	Proteobacteri	Alphaproteob	Sphingomoni	Sphingomoni	Novosphing	99.59	TRUE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Pantoea sp. 111	111	Proteobacteri	Gammaprote	Enterobacteri	Enterobacteri	Pantoea	100	TRUE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Inocul.
Pseudomonas sp. 8O	8O	Proteobacteri	Gammaprote	Pseudomonas	Pseudomonas	Pseudomonas	99.7727	TRUE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Inocul.
Rhizobium sp. EC-SD404	404	Proteobacteri	Alphaproteob	Rhizobiales	Rhizobiaceae	Rhizobium	100	TRUE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Inocul.

Table S2. Overview of the growth rate of selected bacteria at different concentrations of examined antibiotics in this study.

CHL (125µl/25ml)		o.3 µg/ml		0.6 µg/ml		1.2 µg/ml		2.4 µg/ml		4.8 µg/ml		9.6 µg/ml															
Strains	ID																										
Micrococcus sp. 116	116	2		2		4		3		4		3															
Aeromicrobium sp. 9AM	9AM	2		2		4		3		4		3															
Pseudoclavibacter sp. 8L	8L	2		2		4		3		4		3															
Frigoribacterium sp. 9N	9N	2		2		4		3		4		3															
Maribacter sp. 151	151	2		2		4		3		4		3															
Flavobacterium sp. 9R	9R	2		2		4		3		4		3															
Flavobacterium sp. 9AF	9AF									4		3															
Bacillus sp. 9J	9J	2		2		4		3		4		3															
Erythrobacter sp. EC-F	430	2		2								3															
Rhizobium sp. EC-SD	404	3		3		4		3		4		3															
Pantoea sp. 111	111	2		2		4		3		4		3															
Novosphingobium sp. 9U	9U									4		3															
Aeromonas sp. 9A	9A	2		2		4		3		4		3															
Pseudomonas sp. 8O	8O	2		2		4		3		4		3															
Aeromonas sp. 8C	8C	2		2						4		3															
NEO (31.5µl/25ml)		0.39 µg/ml		0.78 µg/ml		1.57 µg/ml		3.14 µg/ml		3.93 µg/ml		7.87 µg/ml		11.8 µg/ml		15.7 µg/ml		19.68 µg/ml		24.8 µg/ml							
Strains	ID	0.5µl		1µl		2µl		4µl		5µl		10µl		15µl		20µl		25µl		31.5µl							
Micrococcus sp. 116	116									3																	
Aeromicrobium sp. 9AM	9AM									4																	
Pseudoclavibacter sp. 8L	8L											2				3									3		
Frigoribacterium sp. 9N	9N									4																	
Maribacter sp. 151	151											2				3									3		
Flavobacterium sp. 9R	9R	6		7		5		3		3		4		4		5				0					0		
Flavobacterium sp. 9AF	9AF											2				3									3		
Bacillus sp. 9J	9J									4																	
Erythrobacter sp. EC-F	430																										
Rhizobium sp. EC-SD	404																										
Pantoea sp. 111	111																										
Novosphingobium sp. 9U	9U											2				3									3		
Aeromonas sp. 9A	9A											5				3											
Pseudomonas sp. 8O	8O											5				3											
Aeromonas sp. 8C	8C											2				3									3		
CHL+NEO																											
Strains	ID	5days		7days		13 days		15days		17days																	
Micrococcus sp. 116	116			Yes																							
Aeromicrobium sp. 9AM	9AM			Yes																							
Pseudoclavibacter sp. 8L	8L			Yes																							
Frigoribacterium sp. 9N	9N			Yes																							
Maribacter sp. 151	151			Yes																							
Flavobacterium sp. 9R	9R					Yes																					
Flavobacterium sp. 9AF	9AF			Yes																							
Bacillus sp. 9J	9J							Yes																			
Erythrobacter sp. EC-F	430			Yes																							
Rhizobium sp. EC-SD	404			Yes																							
Pantoea sp. 111	111			Yes																							
Novosphingobium sp. 9U	9U					Yes																					
Aeromonas sp. 9A	9A			Yes																							
Pseudomonas sp. 8O	8O			Yes																							
Aeromonas sp. 8C	8C							Yes																			

495 **Table S3:** Sample metadata

SampleID	Culture	Replicate	Time series	Temperature	Treatment	CUTim	CuT	CuTimTr	
T0.C1.1.1	ATB	R1	t0	16OC	Control	t0-C	ATB-C	ATB-t0-C	
T0.C1.2.1	ATB	R2	t0	16OC	Control	t0-C	ATB-C	ATB-t0-C	
T0.C1.3.1	ATB	R3	t0	16OC	Control	t0-C	ATB-C	ATB-t0-C	
T0.C1.4.1	ATB	R4	t0	16OC	Control	t0-C	ATB-C	ATB-t0-C	
T0.C2.1.1	NATUR	R1	t0	16OC	Control	t0-C	NATUR-C	NATUR-t0-C	
T0.C2.2.1	NATUR	R2	t0	16OC	Control	t0-C	NATUR-C	NATUR-t0-C	
T0.C2.3.1	NATUR	R3	t0	16OC	Control	t0-C	NATUR-C	NATUR-t0-C	
T0.C2.4.1	NATUR	R4	t0	16OC	Control	t0-C	NATUR-C	NATUR-t0-C	
T0.T1.1	INOCUL	R1	t0	16OC	Control	t0-C	INOCUL-C	INOCUL-t0-C	
T0.T2.1	INOCUL	R2	t0	16OC	Control	t0-C	INOCUL-C	INOCUL-t0-C	
T0.T3.1	INOCUL	R3	t0	16OC	Control	t0-C	INOCUL-C	INOCUL-t0-C	
T0.T4.1	INOCUL	R4	t0	16OC	Control	t0-C	INOCUL-C	INOCUL-t0-C	
T10.C1.5.1	ATB	R1	t10	16OC	Control	t10-C	ATB-C	ATB-t10-C	
T10.C1.6.1	ATB	R1	t10	16OC	Control	t10-C	ATB-C	ATB-t10-C	
T10.C1.7.1	ATB	R1	t10	16OC	Control	t10-C	ATB-C	ATB-t10-C	
T10.C1.8.1	ATB	R1	t10	16OC	Control	t10-C	ATB-C	ATB-t10-C	
T10.C2.5.1	NATUR	R1	t10	16OC	Control	t10-C	NATUR-C	NATUR-t10-C	
T10.C2.6.1	NATUR	R1	t10	16OC	Control	t10-C	NATUR-C	NATUR-t10-C	
T10.C2.7.1	NATUR	R1	t10	16OC	Control	t10-C	NATUR-C	NATUR-t10-C	
T10.C2.8.1	NATUR	R1	t10	16OC	Control	t10-C	NATUR-C	NATUR-t10-C	
T10.T5.1	INOCUL	R1	t10	16OC	Control	t10-C	INOCUL-C	INOCUL-t10-C	
T10.T6.1	INOCUL	R1	t10	16OC	Control	t10-C	INOCUL-C	INOCUL-t10-C	
T10.T7.1	INOCUL	R1	t10	16OC	Control	t10-C	INOCUL-C	INOCUL-t10-C	
T10.T8.1	INOCUL	R1	t10	16OC	Control	t10-C	INOCUL-C	INOCUL-t10-C	
T10.C1.5.2	ATB	R2	t14	16OC	Control	t14-C	ATB-C	ATB-t14-C	
T10.C1.6.2	ATB	R2	t14	16OC	Control	t14-C	ATB-C	ATB-t14-C	
T10.C1.7.2	ATB	R2	t14	16OC	Control	t14-C	ATB-C	ATB-t14-C	
T10.C1.8.2	ATB	R2	t14	16OC	Control	t14-C	ATB-C	ATB-t14-C	
T10.C2.5.2	NATUR	R2	t14	16OC	Control	t14-C	NATUR-C	NATUR-t14-C	
T10.C2.6.2	NATUR	R2	t14	16OC	Control	t14-C	NATUR-C	NATUR-t14-C	
T10.C2.7.2	NATUR	R2	t14	16OC	Control	t14-C	NATUR-C	NATUR-t14-C	
T10.C2.8.2	NATUR	R2	t14	16OC	Control	t14-C	NATUR-C	NATUR-t14-C	
T10.T5.2	INOCUL	R2	t14	16OC	Control	t14-C	INOCUL-C	INOCUL-t14-C	
T10.T6.2	INOCUL	R2	t14	16OC	Control	t14-C	INOCUL-C	INOCUL-t14-C	
T10.T7.2	INOCUL	R2	t14	16OC	Control	t14-C	INOCUL-C	INOCUL-t14-C	
T10.T8.2	INOCUL	R2	t14	16OC	Control	t14-C	INOCUL-C	INOCUL-t14-C	
T14.C1.1.2	ATB	R1	t14	20OC	Stress	t14-S	ATB-S	ATB-t14-S	
T14.C1.2.2	ATB	R2	t14	20OC	Stress	t14-S	ATB-S	ATB-t14-S	
T14.C1.3.2	ATB	R3	t14	20OC	Stress	t14-S	ATB-S	ATB-t14-S	
T14.C1.4.2	ATB	R4	t14	20OC	Stress	t14-S	ATB-S	ATB-t14-S	
T14.C2.1.2	NATUR	R1	t14	20OC	Stress	t14-S	NATUR-S	NATUR-t14-S	
T14.C2.2.2	NATUR	R2	t14	20OC	Stress	t14-S	NATUR-S	NATUR-t14-S	
T14.C2.3.2	NATUR	R3	t14	20OC	Stress	t14-S	NATUR-S	NATUR-t14-S	
T14.T1.2	INOCUL	R1	t14	20OC	Stress	t14-S	INOCUL-S	INOCUL-t14-S	
T14.T2.2	INOCUL	R2	t14	20OC	Stress	t14-S	INOCUL-S	INOCUL-t14-S	
T14.T3.2	INOCUL	R3	t14	20OC	Stress	t14-S	INOCUL-S	INOCUL-t14-S	
T14.T4.2	INOCUL	R4	t14	20OC	Stress	t14-S	INOCUL-S	INOCUL-t14-S	
T10.C1.5.3	ATB	R3	t16	16OC	Control	t16-C	ATB-C	ATB-t16-C	
T10.C1.6.3	ATB	R3	t16	16OC	Control	t16-C	ATB-C	ATB-t16-C	
T10.C1.7.3	ATB	R3	t16	16OC	Control	t16-C	ATB-C	ATB-t16-C	
T10.C1.8.3	ATB	R3	t16	16OC	Control	t16-C	ATB-C	ATB-t16-C	
T10.C2.5.3	NATUR	R3	t16	16OC	Control	t16-C	NATUR-C	NATUR-t16-C	
T10.C2.6.3	NATUR	R3	t16	16OC	Control	t16-C	NATUR-C	NATUR-t16-C	
T10.C2.7.3	NATUR	R3	t16	16OC	Control	t16-C	NATUR-C	NATUR-t16-C	
T10.C2.8.3	NATUR	R3	t16	16OC	Control	t16-C	NATUR-C	NATUR-t16-C	
T10.T5.3	INOCUL	R3	t16	16OC	Control	t16-C	INOCUL-C	INOCUL-t16-C	
T10.T6.3	INOCUL	R3	t16	16OC	Control	t16-C	INOCUL-C	INOCUL-t16-C	
T10.T7.3	INOCUL	R3	t16	16OC	Control	t16-C	INOCUL-C	INOCUL-t16-C	
T10.T8.3	INOCUL	R3	t16	16OC	Control	t16-C	INOCUL-C	INOCUL-t16-C	
T16.C1.1.3	ATB	R1	t16	22OC	Stress	t16-S	ATB-S	ATB-t16-S	
T16.C1.2.3	ATB	R2	t16	22OC	Stress	t16-S	ATB-S	ATB-t16-S	
T16.C1.3.3	ATB	R3	t16	22OC	Stress	t16-S	ATB-S	ATB-t16-S	
T16.C1.4.3	ATB	R4	t16	22OC	Stress	t16-S	ATB-S	ATB-t16-S	
T16.C2.1.3	NATUR	R1	t16	22OC	Stress	t16-S	NATUR-S	NATUR-t16-S	
T16.C2.2.3	NATUR	R2	t16	22OC	Stress	t16-S	NATUR-S	NATUR-t16-S	
T16.C2.3.3	NATUR	R3	t16	22OC	Stress	t16-S	NATUR-S	NATUR-t16-S	
T16.C2.4.3	NATUR	R4	t16	22OC	Stress	t16-S	NATUR-S	NATUR-t16-S	
T16.T1.3	INOCUL	R1	t16	22OC	Stress	t16-S	INOCUL-S	INOCUL-t16-S	
T16.T2.3	INOCUL	R2	t16	22OC	Stress	t16-S	INOCUL-S	INOCUL-t16-S	
T16.T3.3	INOCUL	R3	t16	22OC	Stress	t16-S	INOCUL-S	INOCUL-t16-S	
T16.T4.3	INOCUL	R4	t16	22OC	Stress	t16-S	INOCUL-S	INOCUL-t16-S	

497 **Table S4:** Alpha diversity indices

SampleID	observ	diversity_inverse_simp	diversity_shannon	evenness_pielou	evenness_simpson	dominance_simpson	Samples	Culture	Replicate	Time	serik	Treatment	diversity_.
T0.C1.1.1	209	65.78122375	4.635674766	0.867724584	0.314742697	0.015201906	T0.C1.1.1	ATB	R1	t0		Control	4.63567
T0.C1.2.1	255	38.48769115	4.226029821	0.762647325	0.150932122	0.025982333	T0.C1.2.1	ATB	R2	t0		Control	4.22603
T0.C1.3.1	288	41.2517411	4.217438891	0.744741007	0.143235212	0.0242421401	T0.C1.3.1	ATB	R3	t0		Control	4.21744
T0.C1.4.1	298	74.5814328	4.728967766	0.830066731	0.250273264	0.013408163	T0.C1.4.1	ATB	R4	t0		Control	4.72897
T0.C2.1.1	225	44.74440875	4.23407198	0.781756553	0.198864039	0.022349161	T0.C2.1.1	NATUR	R1	t0		Control	4.23407
T0.C2.2.1	238	45.12952927	4.300670806	0.785902427	0.189619871	0.022158441	T0.C2.2.1	NATUR	R2	t0		Control	4.30067
T0.C2.3.1	179	41.24542802	4.172227154	0.804302458	0.230421386	0.024245111	T0.C2.3.1	NATUR	R3	t0		Control	4.17223
T0.C2.4.1	177	41.15570958	4.151394901	0.802023727	0.232518133	0.024297965	T0.C2.4.1	NATUR	R4	t0		Control	4.15139
T0.T1.1	251	69.72233233	4.704260973	0.851380154	0.277778216	0.014342607	T0.T1.1	INOCUL	R1	t0		Control	4.70426
T0.T2.1	266	46.00625713	4.438163262	0.794871711	0.172955854	0.021736174	T0.T2.1	INOCUL	R2	t0		Control	4.43816
T0.T3.1	164	53.64678016	4.473598783	0.877199206	0.327114513	0.018640448	T0.T3.1	INOCUL	R3	t0		Control	4.4736
T0.T4.1	174	90.64201747	4.7773896	0.926020235	0.520931135	0.011032411	T0.T4.1	INOCUL	R4	t0		Control	4.77739
T10.C1.5.1	177	33.74147097	3.914423733	0.756242368	0.19062978	0.029637119	T10.C1.5.1	ATB	R1	t10		Control	3.91442
T10.C1.5.2	98	28.07568219	3.592672977	0.783576545	0.286486553	0.035618013	T10.C1.5.2	ATB	R2	t14		Control	3.59267
T10.C1.5.3	135	27.29934797	3.56516948	0.726803215	0.202217392	0.036630912	T10.C1.5.3	ATB	R3	t16		Control	3.56517
T10.C1.6.1	136	26.24648506	3.637250204	0.740383823	0.192988861	0.03810034	T10.C1.6.1	ATB	R1	t10		Control	3.63725
T10.C1.6.2	157	29.05649781	3.85749491	0.762916808	0.185073234	0.03441571	T10.C1.6.2	ATB	R2	t14		Control	3.85749
T10.C1.6.3	93	22.96305467	3.418635611	0.754232889	0.246914566	0.043548213	T10.C1.6.3	ATB	R3	t16		Control	3.41864
T10.C1.7.1	102	23.85773713	3.499143526	0.75657602	0.233899364	0.041915124	T10.C1.7.1	ATB	R1	t10		Control	3.49914
T10.C1.7.2	79	25.90684317	3.454466107	0.790595568	0.327934724	0.03859984	T10.C1.7.2	ATB	R2	t14		Control	3.45447
T10.C1.7.3	116	26.25661718	3.491088725	0.734410958	0.226350148	0.038058637	T10.C1.7.3	ATB	R3	t16		Control	3.49109
T10.C1.8.1	79	26.21848815	3.481378962	0.796754894	0.331879597	0.038141025	T10.C1.8.1	ATB	R1	t10		Control	3.48138
T10.C1.8.2	43	24.86083096	3.350589429	0.890829875	0.578158859	0.040223917	T10.C1.8.2	ATB	R2	t14		Control	3.35059
T10.C1.8.3	126	26.05771462	3.53113836	0.730134518	0.206807259	0.038376351	T10.C1.8.3	ATB	R3	t16		Control	3.53114
T10.C2.5.1	212	74.28521389	4.632241655	0.864774955	0.350401952	0.013461629	T10.C2.5.1	NATUR	R1	t10		Control	4.63224
T10.C2.5.2	189	83.92979251	4.697185043	0.896110596	0.444072976	0.01191472	T10.C2.5.2	NATUR	R2	t14		Control	4.69719
T10.C2.5.3	225	80.11557242	4.710398875	0.86970302	0.356069211	0.012481968	T10.C2.5.3	NATUR	R3	t16		Control	4.7104
T10.C2.6.1	213	74.69027018	4.606759794	0.859262963	0.350658545	0.013388625	T10.C2.6.1	NATUR	R1	t10		Control	4.60676
T10.C2.6.2	174	66.37871193	4.548269015	0.88160889	0.38148685	0.015065071	T10.C2.6.2	NATUR	R2	t14		Control	4.54827
T10.C2.6.3	249	78.98156023	4.700432287	0.851920691	0.317195021	0.012661183	T10.C2.6.3	NATUR	R3	t16		Control	4.70043
T10.C2.7.1	211	69.2924844	4.650856196	0.869017093	0.3284004	0.01443158	T10.C2.7.1	NATUR	R1	t10		Control	4.65086
T10.C2.7.2	184	80.99832624	4.673556145	0.896186715	0.440208295	0.012345934	T10.C2.7.2	NATUR	R2	t14		Control	4.67356
T10.C2.7.3	218	85.55253821	4.7562912	0.883330961	0.392442836	0.011688724	T10.C2.7.3	NATUR	R3	t16		Control	4.75629
T10.C2.8.1	190	74.89523757	4.62209168	0.880897746	0.394185461	0.013351984	T10.C2.8.1	NATUR	R1	t10		Control	4.62209
T10.C2.8.2	180	80.36303348	4.632489935	0.892071717	0.446461297	0.012443532	T10.C2.8.2	NATUR	R2	t14		Control	4.63249
T10.C2.8.3	137	84.05821921	4.628136766	0.940681851	0.613563644	0.011896517	T10.C2.8.3	NATUR	R3	t16		Control	4.62814
T10.T5.1	219	47.03336789	4.210444067	0.781293009	0.214764237	0.021261501	T10.T5.1	INOCUL	R1	t10		Control	4.21044
T10.T5.2	154	49.00811558	4.181015705	0.830068503	0.318234517	0.020404784	T10.T5.2	INOCUL	R2	t14		Control	4.18102
T10.T5.3	308	40.03152867	4.197199523	0.732482798	0.129972496	0.02498031	T10.T5.3	INOCUL	R3	t16		Control	4.1972
T10.T6.1	186	33.84163918	3.971480712	0.759983398	0.181944297	0.029549396	T10.T6.1	INOCUL	R1	t10		Control	3.97148
T10.T6.2	223	40.64345664	4.136779158	0.765054142	0.182257653	0.024604206	T10.T6.2	INOCUL	R2	t14		Control	4.13678
T10.T6.3	276	37.22721221	4.00881605	0.713261589	0.134881204	0.026862071	T10.T6.3	INOCUL	R3	t16		Control	4.00882
T10.T7.1	185	31.58692654	3.871084439	0.74153651	0.170740143	0.031658667	T10.T7.1	INOCUL	R1	t10		Control	3.87108
T10.T7.2	160	35.14782616	3.936141947	0.77556791	0.219673913	0.028451262	T10.T7.2	INOCUL	R2	t14		Control	3.93614
T10.T7.3	93	39.43890971	3.944210131	0.870187215	0.424074258	0.025355671	T10.T7.3	INOCUL	R3	t16		Control	3.94421
T10.T8.1	166	42.21993156	4.083087617	0.798727968	0.254336937	0.023685496	T10.T8.1	INOCUL	R1	t10		Control	4.08309
T10.T8.2	132	45.36915441	4.136098168	0.84707474	0.343705715	0.022041407	T10.T8.2	INOCUL	R2	t14		Control	4.1361
T10.T8.3	91	48.60877442	4.072871344	0.902903613	0.534162356	0.020572417	T10.T8.3	INOCUL	R3	t16		Control	4.07287
T14.C1.1.2	139	26.6325972	3.522960835	0.713948616	0.191601419	0.037547971	T14.C1.1.2	ATB	R1	t14		Stress	3.52296
T14.C1.2.2	179	27.10209912	3.553855546	0.685095669	0.151408375	0.036897511	T14.C1.2.2	ATB	R2	t14		Stress	3.55386
T14.C1.3.2	91	26.02346602	3.466769225	0.768538506	0.285972154	0.038426887	T14.C1.3.2	ATB	R3	t14		Stress	3.46677
T14.C1.4.2	56	23.4850157	3.335479527	0.828618164	0.41937528	0.042580342	T14.C1.4.2	ATB	R4	t14		Stress	3.33548
T14.C2.1.2	272	75.26543255	4.674614986	0.833888698	0.276711149	0.013286312	T14.C2.1.2	NATUR	R1	t14		Stress	4.67461
T14.C2.2.2	217	62.60492596	4.601035183	0.855227318	0.288501963	0.015973184	T14.C2.2.2	NATUR	R2	t14		Stress	4.60104
T14.C2.3.2	155	74.61757064	4.561388604	0.904422788	0.481403682	0.013401669	T14.C2.3.2	NATUR	R3	t14		Stress	4.56139
T14.T1.2	190	53.79228637	4.281279434	0.81594431	0.283117297	0.018590026	T14.T1.2	INOCUL	R1	t14		Stress	4.28128
T14.T2.2	189	56.61200628	4.306787689	0.821632115	0.299534425	0.017664098	T14.T2.2	INOCUL	R2	t14		Stress	4.30679
T14.T3.2	189	40.99063856	4.114243293	0.784899249	0.216881686	0.024395814	T14.T3.2	INOCUL	R3	t14		Stress	4.11424
T14.T4.2	233	49.92241343	4.345744892	0.797232478	0.214259285	0.020031083	T14.T4.2	INOCUL	R4	t14		Stress	4.34574
T16.C1.1.3	90	28.25051255	3.597787761	0.799542208	0.313894584	0.035397588	T16.C1.1.3	ATB	R1	t16		Stress	3.59779
T16.C1.2.3	78	26.81241268	3.49944082	0.803231113	0.343748881	0.037296159	T16.C1.2.3	ATB	R2	t16		Stress	3.49944
T16.C1.3.3	84	24.28141145	3.463833842	0.781759662	0.289064422	0.041183767	T16.C1.3.3	ATB	R3	t16		Stress	3.46383
T16.C1.4.3	140	28.88896355	3.697664293	0.74826626	0.20634974	0.034615295	T16.C1.4.3	ATB	R4	t16		Stress	3.69766
T16.C2.1.3	204	79.81586584	4.683226149	0.880616864	0.391254244	0.012528837	T16.C2.1.3	NATUR	R1	t16		Stress	4.68323
T16.C2.2.3	186	77.55819577	4.679194648	0.895411688	0.416979547	0.012893544	T16.C2.2.3	NATUR	R2	t16		Stress	4.67919
T16.C2.3.3	160	82.08859129	4.651729504	0.916565555	0.513053696	0.012181961	T16.C2.3.3	NATUR	R3	t16		Stress	4.65173
T16.C2.4.3	148	79.66507831	4.632648617	0.927046594	0.538277556	0.012552552	T16.C2.4.3	NATUR	R4	t16		Stress	4.63265
T16.T1.3	147	50.17991874	4.185223671	0.838649475	0.341359991	0.019928291	T16.T1.3	INOCUL	R1	t16		Stress	4.18522
T16.T2.3	139	48.8280097	4.161652927	0.843383304	0.351280645	0.020480048	T16.T2.3	INOCUL	R2	t16		Stress	4.16165
T16.T3.3	146	42.16212629	4.111702288	0.825045514	0.288781687	0.023717969	T16.T3.3	INOCUL	R3	t16		Stress	4.1117
T16.T4.3	160	43.49963987	4.204619415	0.828468062	0.271872749	0.022988696	T16.T4.3	INOCUL	R4	t16		Stress	4.20462

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501 **Table S5: NSTI values**

SampleID	Culture	Replicate	Time series	Treatment	NSTI
T0.C1.1.1	ATB	R1	t0	Control	0.05677776
T0.C1.2.1	ATB	R2	t0	Control	0.0480
T0.C1.3.1	ATB	R3	t0	Control	0.0496
T0.C1.4.1	ATB	R4	t0	Control	0.05315927
T0.C2.1.1	NATUR	R1	t0	Control	0.04720949
T0.C2.2.1	NATUR	R2	t0	Control	0.05315927
T0.C2.3.1	NATUR	R3	t0	Control	0.04734416
T0.C2.4.1	NATUR	R4	t0	Control	0.04673616
T0.T1.1	INOCUL	R1	t0	Control	0.0553017
T0.T2.1	INOCUL	R2	t0	Control	0.05546127
T0.T3.1	INOCUL	R3	t0	Control	0.07043085
T0.T4.1	INOCUL	R4	t0	Control	0.06053751
T10.C1.5.1	ATB	R1	t10	Control	0.04864208
T10.C1.5.2	ATB	R2	t14	Control	0.04340053
T10.C1.5.3	ATB	R3	t16	Control	0.04382899
T10.C1.6.1	ATB	R1	t10	Control	0.04341665
T10.C1.6.2	ATB	R2	t14	Control	0.04851226
T10.C1.6.3	ATB	R3	t16	Control	0.04374442
T10.C1.7.1	ATB	R1	t10	Control	0.04311913
T10.C1.7.2	ATB	R2	t14	Control	0.04358509
T10.C1.7.3	ATB	R3	t16	Control	0.04304645
T10.C1.8.1	ATB	R1	t10	Control	0.04264456
T10.C1.8.2	ATB	R2	t14	Control	0.04164816
T10.C1.8.3	ATB	R3	t16	Control	0.04409102
T10.C2.5.1	NATUR	R1	t10	Control	0.05293795
T10.C2.5.2	NATUR	R2	t14	Control	0.05307948
T10.C2.5.3	NATUR	R3	t16	Control	0.05537418
T10.C2.6.1	NATUR	R1	t10	Control	0.05197292
T10.C2.6.2	NATUR	R2	t14	Control	0.05423166
T10.C2.6.3	NATUR	R3	t16	Control	0.05245814
T10.C2.7.1	NATUR	R1	t10	Control	0.05202842
T10.C2.7.2	NATUR	R2	t14	Control	0.05386173
T10.C2.7.3	NATUR	R3	t16	Control	0.05369354
T10.C2.8.1	NATUR	R1	t10	Control	0.05183283
T10.C2.8.2	NATUR	R2	t14	Control	0.05072544
T10.C2.8.3	NATUR	R3	t16	Control	0.05721365
T10.T5.1	INOCUL	R1	t10	Control	0.04151147
T10.T5.2	INOCUL	R2	t14	Control	0.04244061
T10.T5.3	INOCUL	R3	t16	Control	0.04461342
T10.T6.1	INOCUL	R1	t10	Control	0.03788673
T10.T6.2	INOCUL	R2	t14	Control	0.0398021
T10.T6.3	INOCUL	R3	t16	Control	0.03852778
T10.T7.1	INOCUL	R1	t10	Control	0.03395527
T10.T7.2	INOCUL	R2	t14	Control	0.03544289
T10.T7.3	INOCUL	R3	t16	Control	0.03448663
T10.T8.1	INOCUL	R1	t10	Control	0.03623997
T10.T8.2	INOCUL	R2	t14	Control	0.03998218
T10.T8.3	INOCUL	R3	t16	Control	0.03943479
T14.C1.1.2	ATB	R1	t14	Stress	0.04345972
T14.C1.2.2	ATB	R2	t14	Stress	0.04259941
T14.C1.3.2	ATB	R3	t14	Stress	0.04323101
T14.C1.4.2	ATB	R4	t14	Stress	0.04309568
T14.C2.1.2	NATUR	R1	t14	Stress	0.05366224
T14.C2.2.2	NATUR	R2	t14	Stress	0.05228219
T14.C2.3.2	NATUR	R3	t14	Stress	0.05131831
T14.T1.2	INOCUL	R1	t14	Stress	0.04079558
T14.T2.2	INOCUL	R2	t14	Stress	0.03898642
T14.T3.2	INOCUL	R3	t14	Stress	0.04335328
T14.T4.2	INOCUL	R4	t14	Stress	0.04521808
T16.C1.1.3	ATB	R1	t16	Stress	0.04490096
T16.C1.2.3	ATB	R2	t16	Stress	0.04253589
T16.C1.3.3	ATB	R3	t16	Stress	0.04382073
T16.C1.4.3	ATB	R4	t16	Stress	0.04592268
T16.C2.1.3	NATUR	R1	t16	Stress	0.05481751
T16.C2.2.3	NATUR	R2	t16	Stress	0.05781305
T16.C2.3.3	NATUR	R3	t16	Stress	0.05333084
T16.C2.4.3	NATUR	R4	t16	Stress	0.05764996
T16.T1.3	INOCUL	R1	t16	Stress	0.04373309
T16.T2.3	INOCUL	R2	t16	Stress	0.03842605
T16.T3.3	INOCUL	R3	t16	Stress	0.04360146
T16.T4.3	INOCUL	R4	t16	Stress	0.04543375

References

- Alejandre-Colomo C, Francis B, Viver T *et al.* Cultivable Winogradskyella species are genomically distinct from the sympatric abundant candidate species. *ISME Communications* 2021;**1**: 51.
- Barbera P, Kozlov AM, Czech L *et al.* EPA-ng: Massively Parallel Evolutionary Placement of Genetic Sequences. *Systematic Biology* 2018;**68**: 365-9.
- Belcour A, Frioux C, Aite M *et al.* Metage2Metabo, microbiota-scale metabolic complementarity for the identification of key species. *eLife* 2020;**9**: e61968.
- Burgunter-Delamare B, KleinJan H, Frioux C *et al.* Metabolic Complementarity Between a Brown Alga and Associated Cultivable Bacteria Provide Indications of Beneficial Interactions. *Frontiers in Marine Science* 2020;**7**: 85.
- Califano G, Wichard T. Preparation of axenic cultures in Ulva (Chlorophyta), DOI 10.1201/b21460-9, 2018, 159-71.
- Callahan BJ, McMurdie PJ, Rosen MJ *et al.* DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;**13**: 581-3.
- Cirri E, Vyverman W, Pohnert G. Biofilm interactions—bacteria modulate sexual reproduction success of the diatom *Seminavis robusta*. *FEMS Microbiol Ecol* 2018;**94**.
- Cock JM, Sterck L, Rouzé P *et al.* The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae. *Nature* 2010;**465**: 617.
- Cottrell MT, Suttle CA. Production of axenic cultures of micromonas pusilla (prasinophyceae) using antibiotic. *Journal of Phycology* 1993;**29**: 385-7.
- Czech L, Barbera P, Stamatakis A. Genesis and Gappa: processing, analyzing and visualizing phylogenetic (placement) data. *Bioinformatics* 2020;**36**: 3263-5.
- Dittami SM, Arboleda E, Auguet J-C *et al.* A community perspective on the concept of marine holobionts: state-of-the-art, challenges, and future directions. *Zendoo, peer-reviewed and recommended by PCI Ecology* 2021, DOI 10.5281/zenodo.3696771.
- Dittami SM, Corre E, Brillet-Guéguen L *et al.* The genome of *Ectocarpus subulatus* – A highly stress-tolerant brown alga. *Marine Genomics* 2020;**52**: 100740.
- Dittami SM, Duboscq-Bidot L, Perennou M *et al.* Host–microbe interactions as a driver of acclimation to salinity gradients in brown algal cultures. *ISME J* 2016;**10**: 51-63.

535 Douglas GM, Maffei VJ, Zaneveld JR *et al.* PICRUSt2 for prediction of metagenome
 536 functions. *Nat Biotechnol* 2020;**38**: 685-8.
 537 Egan S, Harder T, Burke C *et al.* The seaweed holobiont: understanding seaweed–bacteria
 538 interactions. *FEMS Microbiol Rev* 2013;**37**: 462-76.
 539 Foster ZSL, Sharpton TJ, Grünwald NJ. Metacoder: An R package for visualization and
 540 manipulation of community taxonomic diversity data. *PLoS Comp Biol* 2017;**13**:
 541 e1005404.
 542 Frioux C, Fremy E, Trottier C *et al.* Scalable and exhaustive screening of metabolic functions
 543 carried out by microbial consortia. *Bioinformatics* 2018;**34**: i934-i43.
 544 Geng H, Tran-Gyamfi MB, Lane TW *et al.* Changes in the Structure of the Microbial
 545 Community Associated with Nannochloropsis salina following Treatments with
 546 Antibiotics and Bioactive Compounds. *Front Microbiol* 2016;**7**.
 547 Händel N, Schuurmans JM, Brul S *et al.* Compensation of the metabolic costs of antibiotic
 548 resistance by physiological adaptation in Escherichia coli. *Antimicrob Agents*
 549 *Chemother* 2013;**57**: 3752-62.
 550 Johnsen PJ, Gama JA, Harms K. Bacterial evolution on demand. *eLife* 2021;**10**: e68070.
 551 Karimi E, Geslain E, Belcour A *et al.* Robustness analysis of metabolic predictions in algal
 552 microbial communities based on different annotation pipelines. *PeerJ* 2021a;**9**:
 553 e11344.
 554 Karimi E, Geslain E, Belcour A *et al.* Supplementary materials for "Robustness analysis of
 555 metabolic predictions in algal microbial communities based on different annotation
 556 pipelines. In: Zenodo (ed.), DOI 10.5281/zenodo.4656102: 1.1 Edition, 2021b.
 557 Karimi E, Geslain E, KleinJan H *et al.* Genome Sequences of 72 Bacterial Strains Isolated
 558 from Ectocarpus subulatus: A Resource for Algal Microbiology. *Genome Biol Evol*
 559 2019;**12**: 3647-55.
 560 KleinJan H, Frioux C, Califano G *et al.* Insights into the potential for mutualistic and harmful
 561 host–microbe interactions affecting brown alga freshwater acclimation. *Molecular*
 562 *Ecology* 2023;**32**: 703-23.
 563 Krumhansl KA, Okamoto DK, Rassweiler A *et al.* Global patterns of kelp forest change over
 564 the past half-century. *Proc Natl Acad Sci USA* 2016;**113**: 13785-90.
 565 Lahti L, Shetty S, Blake T *et al.* Tools for microbiome analysis in R. Version 1.9. 1URL:
 566 <http://microbiomegithubcom/microbiome>, 2017.
 567 Levy R, Carr R, Kreimer A *et al.* NetCooperate: a network-based tool for inferring host-
 568 microbe and microbe-microbe cooperation. *BMC Bioinform* 2015;**16**: 164-.

569 Logares R, Bråte J, Bertilsson S *et al.* Infrequent marine-freshwater transitions in the
570 microbial world. *Trends Microbiol* 2009;**17**: 414-22.

571 Louca S, Doebeli M. Efficient comparative phylogenetics on large trees. *Bioinformatics*
572 2017;**34**: 1053-5.

573 Lüning K. Temperature tolerance and biogeography of seaweeds: The marine algal flora of
574 Helgoland (North Sea) as an example. *Helgoländer Meeresuntersuchungen* 1984;**38**:
575 305-17.

576 Matuschek E, Brown DFJ, Kahlmeter G. Development of the EUCAST disk diffusion
577 antimicrobial susceptibility testing method and its implementation in routine
578 microbiology laboratories. *Clin Microbiol Infect* 2014;**20**: O255-O66.

579 McLaren M. Silva SSU taxonomic training data formatted for DADA2 (Silva version 138).
580 *Genève: European Organization for Nuclear Research, doi* 2020;**10**.

581 McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive Analysis and
582 Graphics of Microbiome Census Data. *PLoS ONE* 2013;**8**: e61217.

583 Mirarab S, Nguyen N, Warnow T. SEPP: SATé-Enabled Phylogenetic Placement
584 *Biocomputing* 2012, DOI 10.1142/9789814366496_0024, 2012, 247-58.

585 Morita RY. Psychrophilic bacteria. *Bacteriol Rev* 1975;**39**: 144-67.

586 Oksanen J, Blanchet F, Friendly M *et al.* Vegan: Community Ecology Package. R package
587 version 2.5-5. 2019. <https://CRAN.R-project.org/package=vegan>, 2020.

588 Parks DH, Tyson GW, Hugenholtz P *et al.* STAMP: statistical analysis of taxonomic and
589 functional profiles. *Bioinformatics* 2014;**30**: 3123-4.

590 Pedersen M. *Ectocarpus fasciculatus*: marine brownalga requiring kinetin. *Nature* 1968;**218**:
591 776--.

592 Peters AF, Couceiro L, Tsiamis K *et al.* Barcoding of Cryptic Stages of Marine Brown Algae
593 Isolated from Incubated Substratum Reveals High Diversity in Acinetosporaceae
594 (Ectocarpales, Phaeophyceae). *Cryptogamie, Algologie* 2015;**36**: 3-29, 7.

595 Provost EJ, Kelaher BP, Dworjanyn SA *et al.* Climate-driven disparities among ecological
596 interactions threaten kelp forest persistence. *Global Change Biology* 2017;**23**: 353-61.

597 Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014;**30**: 2068-9.

598 Smale DA. Impacts of ocean warming on kelp forest ecosystems. *New Phytologist* 2020;**225**:
599 1447-54.

600 Smith SD. phylosmith: an R-package for reproducible and efficient microbiome analysis with
601 phyloseq-objects. *Journal of Open Source Software* 2019;**4**: 1442.

- Spoerner M, Wichard T, Bachhuber T *et al.* Growth and Thallus Morphogenesis of *Ulva mutabilis* (Chlorophyta) Depends on A Combination of Two Bacterial Species Excreting Regulatory Factors. *Journal of Phycology* 2012;**48**: 1433-47.
- Steneck RS, Graham MH, Bourque BJ *et al.* Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation* 2002;**29**: 436-59.
- Tapia JE, González B, Goulitquer S *et al.* Microbiota influences morphology and reproduction of the brown alga *Ectocarpus* sp. *Front Microbiol* 2016;**7**.
- Thomas F, Dittami SM, Brunet M *et al.* Evaluation of a new primer combination to minimize plastid contamination in 16S rDNA metabarcoding analyses of alga-associated bacterial communities. *Environ Microbiol Rep* 2020;**12**: 30-7.
- Valero-Mora PM. *ggplot2: elegant graphics for data analysis*. Journal of statistical software volume 35, 2010.
- van der Loos LM, Eriksson BK, Falcão Salles J. The Macroalgal Holobiont in a Changing Sea. *Trends Microbiol* 2019;**27**: 635-50.
- von Dassow P, Ogata H, Probert I *et al.* Transcriptome analysis of functional differentiation between haploid and diploid cells of *Emiliania huxleyi*, a globally significant photosynthetic calcifying cell. *Genome Biol* 2009;**10**: R114.
- West J, Kraft G. *Ectocarpus siliculosus* (Dillwyn) Lyngbye from the Hopkins River Falls, Victoria. The first record of a freshwater brown alga in Australia. *Muelleria* 1996;**9**: 29-33.
- Willey JM, Sherwood L, Prescott LM *et al.* *Prescott, Harley, and Klein's Microbiology*: McGraw-Hill Higher Education, 2008.
- Zengler K, Toledo G, Rappé M *et al.* Cultivating the uncultured. *Proc Natl Acad Sci USA* 2002;**99**: 15681-6.