Supplementary Material

Materials and Methods

Study site selection and biofilm substrate deployment

The study area included a previously well-described water quality gradient (3, 17) comprising of 3 inner nearshore (< 10 km from the coast) and 2 outer nearshore (> 30 km) islands in the Whitsunday Islands, Central GBR (Table 1). Daydream, Pine and Double Cone Island (permanent sites of the long-term Reef Plan Marine Monitoring Program) are positioned inner nearshore subjected to higher nutrients and suspended sediments, and Deloraine and Edward Island, which are positioned outer nearshore less exposed to land runoff (3, 8, 15-17). Standard glass microscope slides (75 x 25 mm) were used for biofilm settlement as these allow growth of very similar bacterial communities as those established on natural coral skeleton substrata, as discussed in (18). Initial settlement of bacteria as biofilm communities on different substrate types differs due to the physical properties of the substrata (7, 14), however, the effect of substrate type diminishes and bacterial communities become more similar over time (1, 7). Glass microscope slides were pre-cleaned with 70 % ethanol, rinsed with sterile water and fixed in polyvinyl chloride frames. Three replicate glass slides were deployed at 2 replicate sites (25 m apart) at each of the five islands. By SCUBA, frames were vertically mounted at 6 m water depth (below the lowest tide level) approximately 30 cm from the underlying sediment on steel pickets (covered by zip lock bags to avoid effects from leached iron) and secured by cable ties. Biofilms were developed repeatedly for ~48 d during 2 replicate dry seasons (August, average seasonal temperature 22 °C) and 2 replicate wet seasons (January, average seasonal temperature 29 °C) over the course of two years (2008-2009 and 2009-2010 seasonal cycles). At the end of the study, this setup yielded 6 replicate samples per island and 60 per season (total of 120 samples). After~48 d, biofilms were sampled for subsequent microbial community analyses by carefully scraping off the biofilm

27 material from the glass substrate into cryovials using sterile No. 11 scalpel blades (yield was
28 usually ~4 g), immediately frozen in liquid nitrogen and stored at -80 °C until further
29 processing.
30
31 *Water quality measurements*
32 Water quality here is defined by Chl *a* concentration, total suspended solids (TSS), Secchi
33 depth, dissolved inorganic nitrogen (DIN, the sum of NH_4, NO_2, NO_3), dissolved organic
34 carbon (DOC) and the physical characteristics of temperature and salinity. Water quality
35 samples were obtained monthly between 2008-2010 and analysed as described in detail in (3)
36 and (12). In short, duplicate samples from two water depths at each location per sample time
37 were analysed for DIN, TSS, Chl *a* and salinity. For particulate nutrients and Chl *a* analysis,
38 water samples were collected on pre-combusted glass fibre filters and analysed after acetone
39 extraction. TSS samples were collected on pre-weighed 0.4 µm polycarbonate filters and
40 concentrations were determined gravimetrically. Salinity was determined using a Portasal
41 Model 8410A Salinometer (Guildline). Light was measured with Odyssey light loggers
42 equipped with wiping units as described in Uthicke & Altenrath (2010) and as light permitted,
43 a Secchi disk depth reading was taken at each sampling site.
44
45 *Genomic DNA extraction, PCR amplification, cloning and sequencing*
46 Total DNA was extracted from 0.25 – 0.5 g of the biofilm (wet weight) sample using the
47 MoBio UltraClean Soil Kit (MoBio Laboratories, Solana Beach, CA, USA) according to the
48 manufacturer's protocol with modifications as in Witt et al. (2011a). Bacterial 16S rRNA
49 genes were amplified by PCR using 63F (5′-CAGGCCTAACACATGCAAGTC-3′) and
50 1389R primers (5′-ACGGGCGGTGTGTACAAG-3′) (Sigma-Proligo, The Woodlands, TX,
51 USA) (9). Each biofilm sample was amplified in triplicate 25 µl reactions containing 2.5 µM
52 non-acetylated bovine serum albumin (New England Biolabs, USA), 2 µM (2 mM each)

53 dNTP (Astral Scientific, Australia), 2.5 µM forward primer 63F, 1.25 µM reverse primer

54 1389R, 1 µM $MgCl_2$ (Qiagen), 1.25U HotStar Taq (Qiagen), 2.5 µl HotStar Buffer (Qiagen,

55 Germany) and ~2 ng of template DNA. Amplification was performed with an initial

56 incubation at 95 °C for 15 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min, 72

57 °C for 1.30 min, and a final extension at 72 °C for 10 min.

58 Eight clone libraries of bacterial 16S rRNA genes amplified from DNA extracted from

59 biofilms grown on glass substrate were constructed, and represent one library for each island

60 (excluding Double cone) and season from year 2008-2009. DNA extracted from all six

61 biofilm replicate samples from each island was then subject to PCR. Each sample was run in

62 triplicates. For the construction of the eight clone libraries, replicates from each island were

63 then pooled according to island and season. Pooled samples were purified using the

64 MinELUTE PCR Clean-Up Kit (Qiagen) and cloned using a TOPO-TA Cloning Kit

65 (Invitrogen, USA) according to the manufacturer's instructions. After blue-white screening,

66 colonies were checked for correct insert size using a colony PCR method with the specific

67 sequencing primer 63F. Randomly picked clones were dispersed in Luria-Bertani (LB) media

68 and 10 % glycerol in 96-well plate format and sent to the Australian Genome Research

69 Facility Ltd. (Brisbane, Australia) for purification and sequencing by an ABI3730 XL

70 Automatic DNA Sequencer.

71 *Analysis of clone sequences*

72 Retrieved sequences were edited using Chromas Lite 2.33 (Technelysium Pty Ltd., Australia),

73 saved as fasta files and submitted to the Greengenes NAST Aligner (for alignment of

74 sequences to the Greengenes database) (4). 16S rRNA gene sequences were checked for

75 chimeras using Bellerophon Version 3 (6), and chimeras were excluded from further analysis.

76 The sequences were submitted to the Greengenes batch sequence classifier

77 [http://greengenes.lbl.gov/cgi-bin/nph-classify.cgi] (11), and taxonomic assignments for each

78 sequence were recorded using the NCBI taxonomy system. All sequences were deposited

79 under GenBank Accession numbers: JQ726882-JQ727208.

80

81 *Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis*

82 Bacterial 16S rRNA genes were PCR amplified using the same reaction mixture and

83 conditions outlined for clone libraries, except that fluorescently labelled 5′-Cy-5 63F (Sigma-

84 Aldrich) forward and unlabelled 1389R reverse primers were used as stated in Witt et al.

85 (2011). DNA template was diluted 1:10 in nuclease free water and cycling conditions were 32

86 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1.3 min. After PCR, samples were

87 prepared for T-RFLP analysis according to Witt et al. (2011a). Terminal restriction fragments

88 (T-RFs) were resolved and visualized using the CEQ 8800 Genetic Analysis System

89 (Beckman-Coulter, Fullerton, CA, USA) with a 600 bp size standard (Beckman-Coulter).

90 Replicate samples were compared using the software T-align (13) with a range of 0.5 bp peak

91 area to determine the consensus peaks between duplicates. The relative fluorescence intensity

92 of the peak area of T-RFs was used as a relative abundance measure for T-RFs in further

93 statistical analyses detailed below. For verification and identification of taxonomic identity of

94 T-RFs, purified DNA from individual clones was also subject to PCR as stated above.

95 *Statistical analysis*

96 Bacterial community data (T-RF values) were square root transformed and standardised to

97 relative abundances. Principal Component (PCA) analysis was used to determine whether

98 bacterial assemblages group by location or season. The assemblage dissimilarities between

99 location and season were tested by applying two-way Permutational Multivariate Analysis of

100 Variance (PERMANOVA) based on permutation procedures (9999 permutations) using the

101 Bray-Curtis distance measure and p values derived from Monte-Carlo (p(MC)) simulations.

102 Pairwise t-tests were used as post hoc test. The contributions of each taxon to the total

103 dissimilarities of treatments were analysed using the Similarity Percentage (SIMPER) routine

104 and represented by vectors in the PCA. Double Cone Is. was excluded from the PCA, as

105 according to the water quality data we cannot clearly categorise this as an inner or outer
106 nearshore island.
107 Prior to analysis, environmental water quality data was averaged over sample seasons,
108 locations and years and z-transformed (average = 0, sd = 1) to accommodate different
109 measurement units of these parameters. To determine the relationship between water quality
110 parameters and bacterial communities a distance-based redundancy (dbRDA) analysis was
111 performed. The dbRDA was constrained by the statistically significant explanatory variables
112 from multivariate multiple regression model (DistLM) marginal tests using a Bray-Curtis
113 distance matrix based on permutation procedures (9999 permutations). As this procedure does
114 not discriminate between location (or season) the data from Double Cone Island was
115 included. Principal Component Analysis, PERMANOVA, DistLM and dbRDA (2, 10) were
116 performed using the Primer 6.0 statistical software (2). To further determine significant
117 differences between relative abundances (peak area) of contributing T-RFs, two-way Analysis
118 of Variance (ANOVA) was performed using the Number Cruncher Statistical System 2007
119 statistical software (NCSS, USA) (5).

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