Protocol for seagrass root and sediment bacterial isolations PCR reactions, sanger sequencing and community analysis

1. DNA extraction:

Follow the DNeasy PowerSoil DNA kit (QIAGEN) protocol

2. PCR reaction(Jensen et al., 2007):

Primer: 27F and 1942R

Reaction mixtures (25 μL) contained: 1)2 μL DNA extract;2)15 pmol of each primer ;3) a Ready-To-Go PCR Bead

The amplification reaction step: 1) a denaturation step of 3 min at 94 °C
2)carry out amplification reactions with 35 cycles of: denaturation (25 s, 94 °C), primer annealing (40 s, 54 °C), a primer extension step (1 min 45 s, 72 °C); 3) a final extension step of 7 min at 72 °C ;4) Purify PCR products using the Gel-Out kit

3. 16S Sanger sequencing (send to sequencing company)

Primer: 27F and 1942R

4. Community analysis

4.1 comparing sequencing results using the NCBI BLAST for species identification
4.2 Calculate Jaccard index to obtain the proportion of species shared between seagrass root samples and sediment samples. Achieve Visualisation by DeepVeen plot
4.3 . To explore microbial species for seagrass indicators: use Indecspecies package in R to obtain

the bacterial species that were always present in the root and sediment samples.

4.4 Analysis of differences between seagrass root bacterial communities and sediment bacterial communities: Use RAxML programme for phylogenetic tree building; implement Unweight-Frac analysis to compare the evolutionary differences between the two communities (root and sediment). Do Permanova analysis verify whether there were significant differences in the composition of seagrass root bacterial communities and sediment bacterial communities, and seagrass root fungal communities among different sampling sites. Produce MDS plots to visualise the differences in seagrass root bacterial community composition.

Reference

1. JENSEN, S. I., KÜHL, M. & PRIEMÉ, A. 2007. Different bacterial communities associated with the roots and bulk sediment of the seagrass Zostera marina. *FEMS Microbiology Ecology,* 62**,** 108-117.