Metagenomics Unveils *Posidonia Oceanica* “Banquettes” as a Potential Source of Novel Bioactive Compounds and CAZymes

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Abstract

Background *Posidonia oceanica* is a long-living and very slow-growing marine seagrass endemic to the Mediterranean Sea. It produces large amounts of leaf material and rhizomes, which can reach the shore and build important banks known as “banquettes”. In recent years, interest in the potential uses of these *P. oceanica* banquettes has increased and it was demonstrated that biomass extracts showed antioxidant, antifungal, and antiviral activities. The discovery of new compounds through the culture of microorganisms is limited, and to overcome this limitation, a metagenomic study was performed to investigate the microbial community associated with *P. oceanica* banquettes.

Results The microbial community associated with *P. oceanica* banquettes was dominated by Alphaproteobacteria, Gammaproteobacteria, Bacteroidetes and Cyanobacteria. *Pseudoalteromonas* was the most abundant genus, followed by *Alteromonas*, *Labrencia*, and *Aquimarina*. The metagenome reads were binned and assembled into 23 near-complete metagenome-assembled genomes (MAGs), which belonged to new families of Cyanobacteria, Myxococcota and Granulosicoccaceae and also to the novel genus recently described as Gammaproteobacteria family UBA10353. A comparative analysis with 60 published metagenomes from different environments, including seawater, marine biofilms, soils, corals, sponges and hydrothermal vents, indicated that banquettes have numbers of natural products and Carbohydrate-Active Enzymes (CAZymes) similar to those found for soils and were only surpassed by marine biofilms. New condensation (C) domains in multi-enzymatic non ribosomal peptide synthetases (NRPS) solely found in *P. oceanica* banquettes were recovered; 90 of the 181 found lacked known homologues. Furthermore, new proteins assigned to cellulosome modules and lignocellulose-degrading enzymes were also found.

Conclusions Our results unveiled the diverse microbial composition of *P. oceanica* banquettes and determined that banquettes are a potential source of bioactive compounds and novel enzymes.

Full Text

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Figures

Figure 1

Taxonomic distribution of the microbial community recovered from *Posidonia oceanica* banquettes using different approaches (16S rRNA metabarcoding, 16S rRNA from metagenome shotgun sequencing, reads from metagenome shotgun sequencing and contigs from metagenome shotgun sequencing) at the level of (A) phylum (B) family and (C) genus.

Figure 2

Relative frequencies of the biosynthetic clusters detected in *Posidonia oceanica* banquettes and other environments using antiSMASH (in contigs >5 Kb). Black bars indicate the numbers of clusters detected among the bacterial contigs and grey bars indicate those not classified taxonomically. Pie charts above the bars indicate the relative contribution of each biosynthetic cluster category accordingly to the legend. The number above each pie chart represents the number of biosynthetic clusters.

Figure 3

Keto-acyl synthase (KS) domains in polyketide synthases (PKSs) detected in *Posidonia oceanica* banquettes. A) Classification of KS domains. B) Boxplot of similarities between the KS domains detected in *P. oceanica* and those found in the other environments analyzed in this work. C) Boxplot of similarities between the KS domains detected in each habitat used in this work against proteins in the nr database. D) Taxonomic distribution of KS domains detected in *P. oceanica* banquettes. FAS: domains involved in fatty acid synthesis; HybridKS: biosynthetic assembly lines that include both PKS and NRPS components; Iterative: PKS domains contain the characteristic domains of type I PKSs; Modular: multidomain architecture consisting of multiple sets of modules; PUFA: polyunsaturated fatty acid; Trans: modular PKS operons lacking cognate AT domains.

Figure 4

Condensation (C) domains in nonribosomal peptide synthetases (NRPS) detected in *Posidonia oceanica* banquette contigs (>5 Kb). A) Classification of C domains. B) Boxplot of similarities between the C domains from *P. oceanica* banquettes and those detected in other environments. C) Boxplot of similarities between the C domains from the different environments and proteins in the nr database. D) Taxonomic distribution of C domains detected in *P. oceanica* banquettes. C: condensation domain ; cyc: Cyclization domains; DCL: domains that link an L-amino acid to a growing peptide ending with a D-amino acid; dual: Dual domains catalyze condensation and epimerization; epim: Epimerization domains change the chirality of the last amino acid in the chain from L- to D- amino acid; hybridC: hybrid PKS/NRPS secondary metabolite; LCL: domains that catalyze formation of a peptide bond between two L-amino acids; modAA: involved in the modification of the incorporated amino acid; start: acylate the first amino acid with a fatty acid, polyketide or other molecule.

Figure 5

Classification of secondary metabolite biosynthetic gene clusters in the 23 Metagenome Assembled Genomes (MAGs) recovered from *Posidonia oceanica* banquettes.

Figure 6

Frequencies of the CAZymes detected using dbCAN in the different environments analyzed in this work. The bar graph shows the number of biosynthetic clusters per number of total ORFs per metagenome. Black indicates the number of CAZymes detected among the bacterial fraction and grey indicates CAZymes found in the taxonomically unclassified sequences. Pie charts demonstrate the relative contribution of each CAZyme family to the total.

Figure 7

CAZymes and auxiliary enzymes in *Posidonia oceanica* banquettes. A) Similarities between the CAZyme sequences from the different environments and sequences in the nr database. B) Similarities between the CAZymes detected in *P. oceanica* and those from the other environments C) Similarities between CAZymes from each family detected in *P. oceanica* and sequences in the nr database D) Identification of the 10 CAZyme clusters that were most abundant in *P. oceanica* banquettes.

Figure 8

Classification of CAZyme families in the 23 MAGs recovered from *Posidonia oceanica* banquettes.

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