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Construction of metagenome BAC library from mangrove soil using the incubation method

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Polyketides are structurally and functionally diverse secondary metabolites that are biosynthesized by polyketide synthases (PKSs) using acyl-CoA precursors. Recent studies in the engineering and structural characterization of PKSs have facilitated the use of target enzymes as biocatalysts to produce novel functionally optimized polyketides. These compounds may serve as potential drug leads. In this study, we have attempted to obtain bacterial PKS gene clusters from mangrove soil metagenomic sample by employing bacterial artificial chromosome (BAC) library system and Type-I PKS primer screening method. To construct BAC library carrying metagenomic DNA, we incubated mangrove soil until 7 days with equal volume of 5 different liquid media(N=4). The microbial cells were collected after 0, 1, 3 or 7 days of incubation and then metagenomic DNA was extracted and purified for BAC library construction. Each metagenomic DNA sample was also analyzed for bacterial community structure by illumina sequencer and compared among the samples. As a result, the soil sample incubated with 1/10 Zobell medium for 1 day showed 2 times larger relative abundance of phylum *Actionbacteria* and *Verrucomicrobia* than initial mangrove soil. On the other hand, it was shown that the bacterial community incubated with 1/10 SN medium for 3 days was dominated by genus *Pseudoalteromonas* (30% relative abundance), while they were detected as minor fraction in the initial mangrove soil (<0.5%). This result suggests that our incubation method could be an efficient method to enrich metagenomic DNA for bacterial taxon of interest, because the phylum *Actinobacteria* or genus *Pseudoalteromonas* are widely known to produce various secondary metabolites. In addition, our incubation method enabled us to obtain more bacterial mass from mangrove soil and thereby more stable metagenomic DNA suitable for subsequent preparation of BAC library.