Establishment of sample preparation workflow for metataxonomic analysis of epiphytic microorganism on pneumatophores

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Introduction

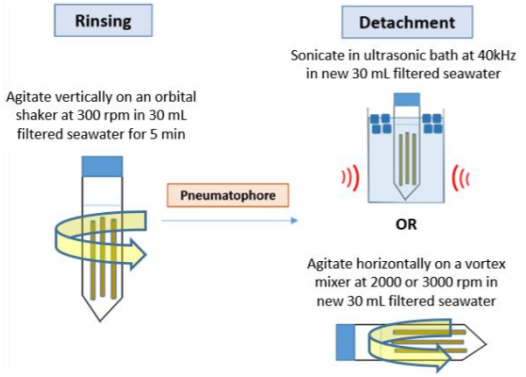
The highly dynamic microhabitats on pneumatophores of mangrove species harbor unique assemblages of microorganisms that are largely unexplored.[1] One big obstacle is the lack of reference methods to collect epiphytic microorganisms from pneumatophores, especially those with strong adherence to withstand tidal flushing. In order to minimize sample preparation bias, a reliable method for collecting epiphytic bacteria from pneumatophores should be established for future studies. To achieve this, we tested the effectiveness of several ways to detach and harvest epiphytic bacterial cells. To date many studies of microbial communities rely on amplicon sequencing and good quality of extracted DNA is crucial.[2,3] We compared the performance of three DNA extraction kits, of which two were for soil due to the presence of soil coatings on all mature pneumatophores, in terms of yield, purity and integrity. Finally, the feasibility of 16S rRNA gene sequencing to analyze the DNA extracted using the developed workflow was confirmed.

Objectives

1) Develop an effective way to detach intact bacterial cells from pneumatophores
2) Maximize the harvest yields of detached bacterial cells
3) Select a DNA extraction kit and lysis condition that produce high-quality DNA
4) Verify the optimized workflow through metataxonomic analysis

Methods

Pneumatophores of *Avicennia marina* were collected from Ting Kok within the same area to minimize spatial variation. All were cut to 8 cm, which was about the same level after 30 mL filtered seawater was added. Three individual roots were subjected to each detachment method in triplicate. Sonication and vortex agitation at two speeds were each subdivided into four groups based on duration and presence of Tween 80, constituting a total of twelve groups.[4]



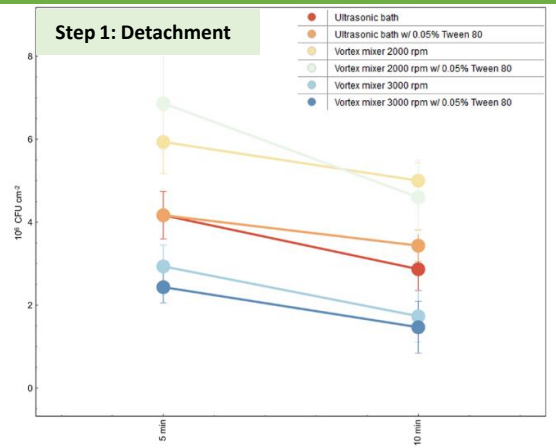
Detached bacteria were enumerated by viable plate count on Marine Agar 2216 and CFUs were expressed per surface area of pneumatophores. The loss of bacterial cells after harvesting by centrifugation and membrane filtration was also enumerated.

Genomic DNA from collected epiphytes was extracted using different kits following manufacturers' protocols. The quantity and quality of extracted DNA were evaluated by NanoDrop 2000c and 1% agarose gels.

16S rRNA gene of region V3-V4 was amplified using specific primer 341F-806R with the barcode.

Results

Step 1: Detachment



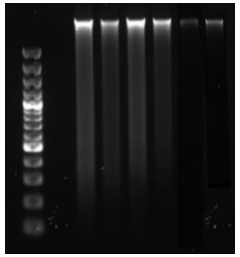
- Under all conditions, prolonged detachment (10 min) caused death in bacteria. **Vortex agitation at 2000 rpm for 5 min with Tween 80 was the most effective approach**, whereas it was too destructive at 3000 rpm.

Step 2: Harvesting

All selected approaches were highlighted.

Harvesting method		% Loss of bacteria (Mean ± SD)
Centrifugation (Pelleting out)	2000 g	16.4 ± 0.57
	7000 g	8.87 ± 0.29
Membrane filtration (0.22μm)	PES	< 0.00001
	MCE	< 0.00001

Step 3: DNA extraction



Lanes 3-8[a]	Yield (μg)	A260/A280	A260/A230
MN L1	2.79	1.89	1.69
MN L2	1.67	1.96	1.11
MN L1 E	2.24	1.91	1.61
MN L2 E	1.61	1.87	1.15
MP Bio	0.49	1.82	0.03
Qiagen	0.62	2.01	1.63

[a] Lane 1 = 100bp DNA ladder; Lane 2 = Negative control; MN = NucleoSpin Soil; MP Bio = FastDNA Spin Kit for Soil; Qiagen = Dneasy Plant Pro; L = Lysis buffer; E = Enhancer

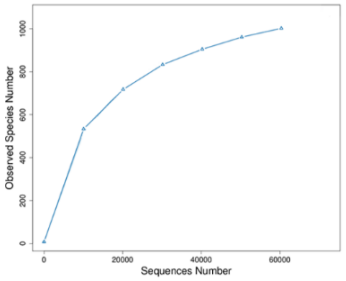
- PES membrane was chosen due to its much lower DNA binding property than MCE.
- NucleoSpin Soil kit using lysis buffer 1 gave the highest yield and purity with intact DNA.

Step 4: DNA Sequencing

Amplicon sequencing QC metrics

Q20 (%)	Q30 (%)	Good's coverage (%)
98.89	95.87	99.6
Chao1	**No. of observed species**	**Shannon index**
1214.2	1002	5.435

Rarefaction curve



References

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Acknowledgement

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