PMA-PCR Method for Discrimination between Live and Dead Marine Microbial Community Members

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Abstract
As a proof of concept, we utilized heat-killed cells of Vibrio AND4 and live cells of Marinobacter algicola in a mix spiked with various concentration of a DNA intercalating, PCR-inhibiting, dye to assess the possibility of selectively inhibiting the PCR amplification of heat-shocked (dead) cells. We incubated each strain of bacteria with PMA in light and in the presence of complex matrices (Pyrite particles and Glass Beads) to simulate the complexity of environmental samples. We hypothesized that following total DNA extraction and 16S rDNA amplification, restriction endonuclease digestion (PMA) and Bst YI would allow us to qualitatively assess the live/dead discriminatory efficiency of PMA on marine cells in complex matrices.

Introduction
Microbial communities in nature are composed of dead and living organisms. For diversity analysis, most environmental microbiological surveys gather total DNA and amplify the 16S rDNA gene. Researchers have utilized RNA as a marker for the determination of the living fraction of organisms in microbial communities. This technique, however, is ineffective for prokaryotes that may be metabolically inactive but completely viable, especially in extremely oligotrophic environments, such as the deep biosphere. In our method of diversity analysis, we focus on the integrity of membrane structure as the key determinant for dead vs. live microbes. We employ Propidium Monoazide (PMA), a DNA-interchelating dye capable of forming a covalent bond between DNA strands of damaged cells inhibiting downstream PCR amplification. By observing the relative abundance of DNA of live cells after PCR amplification, we can determine which marine cells are viable.

Materials and Methods

1. Grow Bacteria
2. Add PMA
3. PCR Amplification
4. DNA Extraction
5. DNA Quantification
6. Restriction Digest
7. Electrolysis
8. Electrophoresis

Results

Marinobacter algicola

<table>
<thead>
<tr>
<th>Sample</th>
<th>M. agar</th>
<th>LB agar</th>
<th>tntc</th>
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</thead>
<tbody>
<tr>
<td>E-4</td>
<td>291</td>
<td>217</td>
<td>152</td>
</tr>
<tr>
<td>E-5</td>
<td>911</td>
<td>458</td>
<td>306</td>
</tr>
<tr>
<td>E-6</td>
<td>204</td>
<td>142</td>
<td>92</td>
</tr>
</tbody>
</table>

Table 2. Cell density quantification of Marinobacter algicola and Vibrio sp. AND4 overnight cultures via serial dilution and 48h plate enumeration in Marine and LB agar.

Restriction Enzyme Map: BccI and Bst YI

Conclusion/Future Work
Restriction digestion with both BccI and BstYI enzyme showed identical band patterns for Marinobacter algicola and Vibrio sp. AND4. This spurious results initially prompted us to check each bacterial culture for possible contamination. Colony morphology observation and fluorescence microscopy analysis confirmed monocultures for both bacterial strains used in this study. The experiment was repeated in triplicate from single colony digestion yielding identical results. Notably, contamination could have also occurred at the molecular level (DNA) prior to 16S rDNA amplification and/or during set up of restriction endonuclease reactions. We suggest the utilization of a cloning vector insert in a competent cell strain. Cloning the 16S rDNA will ensure the amplification of the exclusive small ribosomal sequence and minimize downstream chances of DNA contamination. Lane #9 in figure 3 shows no 16S amplicon was digested or present after PMA treatment of heat-killed cells exclusively. This result shows that PMA was capable of completely inhibiting DNA amplification of cells with heat-damaged membranes.

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References: