Biomineralization of microbes isolated from natural and restored saltmarshes: potential benefits to restoration

James D’Arcy and André Antunes
GEMM-Group for Extreme and Marine Microbiology, Biology Department, Edge Hill University, Ormskirk, UK

Introduction:
Saltmarshes are threatened coastal ecosystems, which are subject to cyclic variation in conditions which create a gradient-rich environment (e.g. salinity, pH). They support very diverse communities of plants and animals, protect the coast from erosion, and play an important role in element cycling and bioremediation [2]. The importance of saltmarshes has only been recently recognised, which has led to significant efforts in restoring sites that had been previously converted to Agriculture. Such restoration attempts have had limited success in returning these sites to their original biodiversity and biological structure. While not yet fully understood, it is thought that several factors are at play, including persisting differences in soil structure and quality [1]. Coastal environments have been previously shown to harbour significant microbial populations capable of producing CaCO3 biominerals. On the other side, CaCO3 biomineral production is known to affect the texture and overall properties of soil, a property currently used in the improvement of soils for Agriculture [3]:

These factors could be linked, and limitations of saltmarsh restoration efforts might result from differences in biomineral production. This study provides an overview on differences between samples that we’ve collected in natural and restored sites, based on cultivation and screening efforts.

Methods:

I. Sampling

Triplicate water and sediment samples were collected from 3 sites at RSPB Marshside (natural), 2 sites at Hesketh Out Marsh West (12-yr restored) and 1 site at Hesketh Out Marsh East (6-month restored).

II. Isolation and purification

Single colonies were isolated in MB/MA through serial dilutions, followed by three passages to achieve an axenic culture.

III. Biomineral screening

Optical microscopy at X40 and X100 magnification and collection of biominerals.

Results:

- 56 axenic strains were isolated from all sites and screened for biomineral production.
- 18 producers were isolated, 9 from each saltmarsh type.
- 8 strains were collected from due to limited incubation period resulting in lack of biomineral growth.

Table 1. Percentage of biomineral producers isolated from natural reference sites (MS1, MS2, MS3, MSsed) and restored saltmarsh sites (HOME1, HOME2, HOMW1, HOMWsed). ND: not determined.

<table>
<thead>
<tr>
<th>Site</th>
<th>Number of strains isolated</th>
<th>Percentage of biomineral producers</th>
<th>Weight of biominerals collected (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS1</td>
<td>4</td>
<td>50%</td>
<td>ND</td>
</tr>
<tr>
<td>MS2</td>
<td>7</td>
<td>14%</td>
<td>7.1</td>
</tr>
<tr>
<td>MS3</td>
<td>8</td>
<td>63%</td>
<td>26.9</td>
</tr>
<tr>
<td>MSsed</td>
<td>6</td>
<td>17%</td>
<td>25.9</td>
</tr>
<tr>
<td>HOME1</td>
<td>4</td>
<td>25%</td>
<td>35.0</td>
</tr>
<tr>
<td>HOMEW1</td>
<td>7</td>
<td>29%</td>
<td>ND</td>
</tr>
<tr>
<td>HOMEW2</td>
<td>13</td>
<td>31%</td>
<td>70.3</td>
</tr>
<tr>
<td>HOMEWsed</td>
<td>7</td>
<td>29%</td>
<td>ND</td>
</tr>
</tbody>
</table>

- Difference in biomineral producers ($W = 8, n = 8, p = >0.05$).
- Difference in Weight of biominerals ($t = -0.62615, df = 6, p = >0.05$).

Discussion:

- Contrary to expectations, no significant differences were observed between natural and restored saltmarshes.
- Potential sources of bias include:
  1. Limitations in extraction method
  2. Likely need for longer incubation period
  3. Limitations from cultivation - development approach
  4. Differences between lab-based and natural environment conditions
- Generated a collection of biomineral producing strains for future studies.
- Some of the strains cultivated are fast biomineral producers and might be relevant for biotechnology.

Future work:

- Further screening of the sites and expansion to other locations.
- Wide-scope approach combining more cultivation conditions with molecular based methods (e.g. metagenomics), and looking into other factors affecting restoration.
- Optimisation of biomineral extraction methods.
- Further studies on current strains such as:
  1. Identifying biomineral producers for use in industry
  2. Screening for other biotechnological applications
  3. Full mineralogical and taxonomic characterisation

Acknowledgements:
We would like to thank the support provided by Andrew Marrist, Faye Schofield, Marta Filipa Simões, Philips Loftus, and Priyanka More. We are also grateful to RSPB Marshside Office Southport for sampling permission, and the Student Opportunity Fund at Edge Hill University for funding support.

References: