Bacterial Degradation of Algal Toxins: Possibilities for Use in Post-Harvest Treatment?

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1) Background
Marine bacteria capable of metabolising algal toxins were isolated. Such bacteria could play a role in elimination of algal toxins from shellfish; possible applications include manipulating the shellfish bacterial flora to depurate algal toxins.

2) Toxin degradation by bacteria
Bacteria were shown to have the capacity to degrade PSP toxins. Bacteria were grown with algal toxins - degradation of the toxins was monitored over time. Results from candidate bacteria for use in depurating shellfish are shown opposite:

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Preliminary id (SSU rRNA)</th>
<th>Total toxicity (STX eq.) degradation (%)¹,²</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD314</td>
<td>Erythrobacter citrus</td>
<td>51</td>
</tr>
<tr>
<td>SD335</td>
<td>Stappia sp.</td>
<td>53</td>
</tr>
<tr>
<td>SD466</td>
<td>Unidentified</td>
<td>55</td>
</tr>
<tr>
<td>SD424</td>
<td>Pseudoalteromonas sp.</td>
<td>60</td>
</tr>
<tr>
<td>SD484</td>
<td>Pseudoalteromonas sp.</td>
<td>67</td>
</tr>
<tr>
<td>SD12</td>
<td>Kibblesia sp.</td>
<td>75</td>
</tr>
</tbody>
</table>

¹Isolates were grown in marine broth with (nM): STX (390), NEO (390), GTX-1 (636), GTX-2 (708), GTX-3 (234) & GTX-4 (210) over 5 d. Change in total PSP toxicity was determined by LC-MS-MS

3) Microencapsulation – delivery of bacteria to shellfish
Microencapsulation of toxin degrading bacteria could be used to deliver a concentrated pulse of bacteria to the digestive system of toxin contaminated shellfish. The size of capsule that could be filtered & ingested by shellfish was determined. Alginate capsules of 3 different sizes (10-50, 50-100 & 100-250µm) containing non-degradable fluorescent microbeads (as a proxy for bacteria) of 3 different colours (blue, pink, yellow) were fed to mussels & scallops (Figs. A, B, C & D).

Both mussels & scallops ingested capsules over 2h. Smaller capsules (blue & pink beads) were readily ingested. A higher proportion of yellow capsules were detected in mussel pseudo-faeces & a higher proportion of blue capsules were detected in scallop pseudo-faeces, the alginate wall was still visible so capsules were not degraded prior to rejection (Fig E).

Pink microbeads were detected in faeces after 16 h with the alginate wall dissolved (Fig. F). The capsules were passed into the digestive system prior to being excreted (Figs. G & H).

4) Detoxifying shellfish – a possibility?
The next stage was to develop a method of depurating shellfish by feeding a diet of microencapsulated bacteria. Bacterial isolate, SD12, shown to degrade PSP toxins in vitro was microencapsulated (50 – 100 μm) (Fig. I) & fed to scallops contaminated with PSP toxins. Empty capsules were used as a control (Fig. J).

Scallops were sampled over time (Fig. K), dissected (Fig. L) and the hepatopancreas (HP) analysed for PSP toxins using LC-MS-MS.

5) Preliminary results
Variation in toxin concentrations in the HP were observed between scallops fed microencapsulated bacteria and empty capsules, although inter-animal variability was high. Fig. M shows example MRM chromatograms for NEO.

Further trials will be undertaken to determine if a practical method for detoxifying cultured shellfish is possible!

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