Sophorolipids Having Enhanced Anti-bacterial Activity

Sophorolipids (SLs) are glycolipids produced fermentatively by yeast such as *Candida bombicola*, *Candida apicola* and *Wickerhamiella domercqiae* and composed of a dimeric sugar linked with a glycosidic bond to a hydroxyl fatty acid. SLs have gained interest in pharmaceutical arena because of its wide array of therapeutic benefits. Shah et al.\(^1\) first reported that natural SLs and its first generation chemical derivatives are efficient microbicidal spermicides having activities similar to Nonoxynol-9. Bluth et al.\(^2\) showed that natural SLs are effective septic shock antagonists. Additionally, SLs have been demonstrated to be effective anticancer agents against cancerous cell lines.\(^3\) Major drawback of the SLs so far has been its cytotoxic activities. While efforts are being made to further chemically modify the molecule to increase its potency and decrease its toxicity, no improvements have been reported. The second generation of SL molecules was generated by conjugating amino acids to fatty acid side chain.\(^4\) However, these molecules were less effective as microbicidal spermicides compared to first generation compounds.

In the current study, we report fermentative production of novel SL molecules that have higher anti-bacterial activity and offer a new backbone for further chemical modifications. Fermentation medium used to produce SLs by *Candida bombicola* has glucose as the primary carbon source. Thus, the SL formed has two glucose molecules. We substituted glucose in the fermentation medium with other sugars described in Table 1. The SLs formed in each of the medium were extracted and purified as per standard protocol.\(^4\) Liquid Chromatography – Mass Spectrometer analyses were carried out for the compounds and the MIC extracts of compounds with mass from 500 – 800 are shown in Figure 1. The peak appearing at 24.5 min in all the chromatograms is because of the
three sophorolipids viz. diacetylated lactonic SL with C18 mono-unsaturated fatty acid [M+H, 689], diacetylated lactonic SL with C18 saturated fatty acid [M+H, 691] and diacetylated open ring SL with mono-unsaturated fatty acid [M+H, 707]. The peak at 25.3 min is also because of the compounds having same mass. Two peaks of the same mass at different retention times have been reported earlier and is attributed to attachment of dimeric sophorose to the hydroxyl group at either the penultimate or terminal fatty acid carbon where the later is the minor component for the regioisomers. The peak at 33.1 min in all chromatogram is because of diacetylated open ring SL with saturated C18 fatty acid [M+H, 709]. New peaks in the region of 35 – 50 min are because of the SL compounds having M+H of 603 and 605 corresponding to unacetylated lactonic SL having di-unsaturated C18 fatty acid and unacetylated lactonic SL having mono-unsaturated C18 fatty acid respectively.

The appearance of new peaks in SLs produced from different sugars in comparison to that produced with glucose in the fermentation medium can be explained because of the presence of different sugar head group in the compound and the inability of the cellular machinery of C. bombicola to acetylate the new sugars. The unacetylated lactonic SLs reaffirms our hypothesis of the presence of new sugar heads in the SLs. Since the acetyl group is added to the glucose in the SL head group by the enzyme acetyl transferase, presence of the unacetylated SLs indicate that the new sugar present in the head group is not glucose. Glucose in the head group of SLs is the natural substrate for acetyl transferase and presence of new sugar head group could be presumed to be composed of different molecules than glucose, on which the enzyme can not act. It is important to note that all the fermentation media had oleic acid as the lipidic substrate and thus no difference in structure could be expected in the fatty acid side chain. Further purification
and characterization studies are needed to understand the precise chemical structure of each SL analog.

Anti-bacterial activity of the purified compounds shows that SLs are more effective against gram +ve bacteria than gram –ve bacteria Table 1. Comparing the anti-bacterial activity of SLs obtained in glucose containing medium, it could be inferred that SLs obtained from different sugar containing mediums vary in their activity against the tested organisms. SLs from arabinose containing medium are more effective against three of the four gram +ve bacteria tested and against Moraxella sp when compared to SLs from glucose containing medium. However, SLs from arabinose shows no inhibition on the growth of E. coli. SLs from lactose containing medium was the most effective compound against B. subtilis.

All the efforts so far to generate a clinically viable compound by chemically modifying the SLs from glucose containing medium have failed, we believe that the molecules generated through the present method will open a new door. With higher activity on its own, these molecules could be hypothesized to be more potent when chemistries similar to that carried out in developing first generation SLs.
Figure 1: LC-MS spectrum of Sophorolipids obtained using different sugar sources in fermentation medium. (A, Glucose; B, Fructose; C, Xylose; D, Ribose; E, Lactose; F, Mannose; G, Arabinose; H, Galactose)
LC-MS experiments were conducted on Shimadzu LCMS- 2010EV. A C8 column (150 x 3.0 mm; Princeton Chromatography Inc.) was utilized as the analytical LC column. Separations were achieved under gradient conditions using 0.05% formic acid in water and 0.05% formic acid in acetonitrile as the mobile phase at a flow rate of 1 mL/min. MS analysis were performed with instrument in positive polarity and APCI interface. Interface temperature was 300°C, CDL temperature was 250°C and heat block at 220°C. Detector voltage was 1.5 kV and scan speed was 4000.
The MLD<sub>50</sub> were determined by a broth microdilution method in 96-well microtiter plates.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Xylose</th>
<th>Ribose</th>
<th>Lactose</th>
<th>Mannose</th>
<th>Arabinose</th>
<th>Galactose</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhodococcus erythropolis</em></td>
<td>0.098</td>
<td>0.024</td>
<td>0.006</td>
<td>0.024</td>
<td>0.098</td>
<td>0.098</td>
<td>0.006</td>
<td>&gt; 6.25</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>0.098</td>
<td>0.098</td>
<td>0.39</td>
<td>0.098</td>
<td>0.024</td>
<td>0.09</td>
<td>0.04</td>
<td>0.39</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>0.098</td>
<td>0.024</td>
<td>0.024</td>
<td>0.098</td>
<td>0.098</td>
<td>0.39</td>
<td>0.04</td>
<td>0.39</td>
</tr>
<tr>
<td><em>Moraxella sp.</em></td>
<td>0.098</td>
<td>0.098</td>
<td>0.024</td>
<td>0.098</td>
<td>0.39</td>
<td>0.098</td>
<td>0.04</td>
<td>0.39</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>&gt; 6.25</td>
<td>&gt; 6.25</td>
<td>&gt; 6.25</td>
<td>&gt; 6.25</td>
<td>&gt; 6.25</td>
<td>&gt; 6.25</td>
<td>NI</td>
<td>NI&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> The MLD<sub>50</sub> were determined by a broth microdilution method in 96-well microtiter plates.

<sup>b</sup> NI – No Inhibition.
References


Vishal Shah*1
Daniel Badia1
Peter Ratsep2

1Department of Biology
Dowling College, Idle Hour Blvd
Oakdale. NY 11769. USA
Phone: (1) 631-244-3339
Fax: (1) 631-244-3003
Email: ShahV@dowling.edu

² Shimadzu Scientific Instruments Inc.
262 D Old New Brunswick Rd,
Piscataway, NJ 08854