Optimization of enzymatic one-pot reactions for production of sialylated HMOs

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Sialylated human milk oligosaccharides

- Human milk oligosaccharides (HMOs) provide numerous health benefits for breast-fed infants.¹
- Sialylated HMOs are of special interest as they supply sialic acids for the development of the infants brain.¹
- The cost-efficient production of sialylated HMOs is of great interest for supplementation of infant formula and for medicinal research.¹

Table 1: Properties of enzymes involved in sialoside production

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Organism</th>
<th>pH</th>
<th>T</th>
<th>K_m [mM]</th>
<th>k_cat [s⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sialic acid aldolase</td>
<td>Escherichia coli</td>
<td>7.5</td>
<td>37</td>
<td>ManNAc: 64.4</td>
<td>10.2</td>
</tr>
<tr>
<td>CMP-sialic acid synthetase</td>
<td>Neisseria meningitidis</td>
<td>8.5</td>
<td>37</td>
<td>NeuAc: 0.34</td>
<td>32</td>
</tr>
<tr>
<td>α-2,3-sialyltransferase</td>
<td>Pasteurella dagmatis</td>
<td>8.0</td>
<td>25</td>
<td>Lactose: 1.5</td>
<td>24</td>
</tr>
</tbody>
</table>

Enzymatic pathway for sialoside production

- Interesting for a cost-efficient production of sialosides is a low cost starting material such as N-acetyl glucosamine.
- Previously studied were one-pot reactions including Sialic acid aldolase, CMP-Sialic acid synthetase and sialyltransferase showing yields between 61 and 99 % depending on sialoside produced.²

Challenges to be met:
- Characterize enzymes in detail
- Analytics

Outlook: One-pot reactions of two or more enzymes

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