Stable Isotope-based in-situ Labelling as a Reference for the Isolation of relevant Styrene Degraders from Biofilters

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Introduction

The enrichment of styrene degrading microorganisms from biofilters resulted in a broad range of degrading isolates with unknown in situ relevance. For this reason an in situ labeling of styrene degrading microorganisms using deuterated styrene and phospholipid fatty acids as biomarkers prior to the isolation approach was performed. By this method the fatty acid profiles of the primary styrene degraders in the biofilter material were determined. The comparison of labeled fatty acids with a fatty acid database gave first evidences for a systematic position of the taxa of interest. The fatty acid profiles of several isolates were compared with the set of labeled fatty acids derived from the original filter material. The correspondence of these biomarkers is a central criterion for the in situ relevance of the isolates. Additionally, the quantitative relevance of the isolates in the filter material was examined by fluorescently labeled rRNA-targeted probes. The combination of isotope-based in-situ-labeling, fluorescence in situ hybridization and appropriate methods allows for an extensive characterization of the primary styrene degraders in the biofilters investigated.

Materials and Methods

Biofilter material was sampled from two different styrene supplied biofilters, one experimental filter with foam-based old glass as support material and an industrial filter with bark-mold. Water content, pH (1M KCl), CFU (minimal-broth media) and temperature were determined. Biofilter material was sampled from two different styrene supplied biofilters, one experimental filter with foam-based old glass and an industrial filter with bark-mold. The microbial community of the biofilter material was characterized by phospholipid fatty acid profiles and fluorescence in situ hybridization. For the in situ labeling of fatty acids of styrene degrading microorganisms, 10g biofilter material was incubated with 2µl deuterated styrene in 500µl broth. The degradation process was followed by gas measurements using GC/MS. After the consumption of all styrene, phospholipids were extracted and the percentage of deuteration of the lipids was determined. Labeled fatty acids were assigned to bacterial genera by a database which contains fatty acid profiles of about 300 genera. Styrene-degrading bacterial isolates were examined for their fatty acid profiles. The variability of these fatty acid profiles was tested for stationary cells on trypticase soy broth (TSB) and minimal-medium (MM) + styrene for certain isolates.

Results

Phospholipid fatty acid profiles and Isotope-labelling

The phospholipid fatty acid profiles of the different filters (fig.1) were almost similar. The main fatty acids are 16:1 cis11 and 16:0. Other fatty acids with higher amounts are 16:1 cis11, 19:0 cyclo 11-12 and in the experimental biofilter 17:1 cis 11-12. Most of these fatty acids showed a detectable percentages of deuteration in the labelling experiment. In both cases, the strongest deuterium is found in 16:1 cis11 and 16:0 (fig.2).

Fluorescence in situ hybridization

The community analyses with FISH also revealed a similar composition of main groups of microorganisms in both filters. Remarkably, taxa with known 16:1 cis11-carrying members like Rhodospirillum or members of gamma-Proteobacteria (family Methylococcaceae), were not present in higher amounts (fig.1).

Isolation of styrene degraders

Until now, there is only one isolate from the investigated biofilters with 16:1 cis11 as a component of its fatty acid profile. However, the amount is low (table 2). As was found in the other examined isolates, its profile is stable and 16:1 cis11 did not increase under certain conditions tested (table 2).

Table 1: Analyses of the biofilter material

<table>
<thead>
<tr>
<th>Biofilter</th>
<th>Filter material</th>
<th>pH</th>
<th>Water content [%]</th>
<th>Organic carbon [%]</th>
<th>(cell wall fat) %</th>
<th>CFU/g dry weight [+/-]</th>
<th>Phospholipid fatty acid profiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental biofilter</td>
<td>(University of Paderborn)</td>
<td>7.5</td>
<td>68.5</td>
<td>7.8</td>
<td>2.8±0.0</td>
<td>2.8±0.0</td>
<td>16:0 20.4</td>
</tr>
<tr>
<td>Industrial biofilter</td>
<td>Bark compost</td>
<td>5.5</td>
<td>87.7</td>
<td>1.7</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>16:0 16.6</td>
</tr>
</tbody>
</table>

Table 2: Fatty acid profiles of the isolates JP1 and T7 under different conditions

<table>
<thead>
<tr>
<th>Isolate</th>
<th>TSB, 3 days, 30°C</th>
<th>MM + styrene, 30°C</th>
<th>TSB, 3 days, 45°C</th>
<th>MM + styrene, 45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>JP1</td>
<td>16:0 20.4</td>
<td>16:1 cis11 45.1</td>
<td>16:0 20.4</td>
<td>16:1 cis11 45.1</td>
</tr>
<tr>
<td>T7</td>
<td>16:0 19.2</td>
<td>16:1 cis11 19.2</td>
<td>16:0 19.2</td>
<td>16:1 cis11 19.2</td>
</tr>
</tbody>
</table>

Conclusions

- Despite their differences in the pH and the support material, a comparable distribution of the main microbial groups was found in both biofilters.
- According to the labelling experiment of phospholipid fatty acids with deuterated styrene, the most active degraders have 16:1 cis11 and 16:0. The high abundances of these fatty acids in the filter profiles suggest that these strains represent the main component of the bacterial biomass.
- The fluorescence in situ hybridization gave no clear indication for the identity of these styrene degrading taxa.
- Until now, only the isolate JP1 shows 16:1 cis11, but in a low amount, which does not increase in stationary cells under certain conditions tested.
- Although several styrene-degrading bacterial strains were isolated from the filter material, these taxa were probably not responsible for the main styrene degrading activity of the biofilter.