The aliphatic amidase AmiE is involved in regulation of Pseudomonas aeruginosa virulence

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### Supplemental material figures and tables.

“The aliphatic amidase AmiE is involved in regulation of *Pseudomonas aeruginosa* virulence”

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**Table S2.** Strains and plasmids used in this study.

<table>
<thead>
<tr>
<th>Strain/Plasmid</th>
<th>Characteristics/modified gene</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA14</td>
<td>Wild type</td>
<td>Rahme <em>et al</em>., 1995</td>
</tr>
<tr>
<td>PA14 AmiE⁺</td>
<td>PA14 over-expressing <em>amiE</em></td>
<td>This study</td>
</tr>
<tr>
<td>PA14 EV</td>
<td>PA14 harboring empty vector</td>
<td>Rosay <em>et al</em> 12</td>
</tr>
<tr>
<td>PA14 ΔamiE</td>
<td>ΔamiE, ID 56575</td>
<td>Liberati <em>et al</em>. 55</td>
</tr>
<tr>
<td>pBBR-MCS5</td>
<td>Cloning Vector</td>
<td>Kovach <em>et al</em>. 56</td>
</tr>
<tr>
<td>pBBR-MCS5::amiE</td>
<td>pBBR-MCS5 harboring <em>amiE</em></td>
<td>This Study</td>
</tr>
</tbody>
</table>

Supplemental Figure S1.
Relative protein amounts in AmiE+ strain (grey histogram) compared to the relative protein amounts in the control strain PA14 EV (white histograms), after 5 h of growth. Quantifications have been obtained from three independent experiments. ★★★ = \( P < 0.001 \); ★★ = \( P < 0.01 \); NS = Not significantly different.

**A**

- **PA14_58730** (Type IV pilin)
- **PA14_63110** (SAM)
- **PA14_51410** (PqcC)
- **PA14_36310** (HcnC)
- **PA14_19130** (RhlI)
- **PA14_51390** (PqsD)
- **PA14_51420** (PqsB)
- **PA14_66620** (PilQ)

**B**
The total cumulative abundance of the protein RhlA was calculated by summing the abundances of only one peptide.

**C**
Proteins showing a differential expression level but not significantly different using the one-way analysis of variance (ANOVA).
Supplemental Figure S2.
Supplemental Figure S3.

PO4 bacteriophage sensitivity assay

$10^7$ bacteria were mixed with ten-fold serial dilutions of bacteriophages ranging from $10^8$ to $10^2$ PFU (plaque forming unit) in 1.5mL of 0.5% Noble Agar and poured on top of 1.5% Agar Luria Bertani medium in a two-compartment Petri dish. After 24h at 37°C, bacterial sensitivity to PO4 bacteriophages was estimated by counting the number of plaques.
Microcolonies formation of *P. aeruginosa* PA14 strains in Artificial Sputum Medium (ASM). Tight microcolonies are visible in light beige at the bottom of the well. The images presented are representative of four independent experiments.

In artificial sputum-like medium, PA14 ΔamiE was able to produce similar microcolonies as PA14 WT, whereas PA14 AmiE+ produced a more diffuse biofilm as compared with the PA14 EV strain.
Supplemental Figure S5.

Culture growth curves (OD\textsubscript{580nm})
Absorbance was recorded every 20 min for 24 hr. Curves are color-coded as follow: PA14 AmiE\textsuperscript{+} strain (orange), PA14 WT (blue), PA14 EV (red) and \textit{ΔamiE} (gray). Results are the mean of twelve replicates from two independent experiments.