Supplementary Materials

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**Fig. S1.** Analysis of AccB production with His$_6$ translational fusion constructs. SCV and Rev1 represent the respective 5´-UTR background. The constructs were expressed in *E. coli* DH5α. Representative picture and graph form three independent blots. Bars represent mean values ± SD relative to wild-type, *P* < 0.05 according to one-tailed Mann-Whitney test.
Fig. S2. Prediction of the secondary structures of DNA oligonucleotides and CD spectroscopy. 

(A) Secondary structure prediction for DNA oligos. The oligos are 27 nucleotides long and represent the 5′-UTR of \textit{accBC}. All secondary structures were predicted with CentroidFold (19). Numbers indicate nucleotide positions relative to A of the ATG start codon of \textit{accB} and color shading indicates base-pairing probabilities from low (0.0) to high (1.0). 

(B) Thermal stability of DNA oligos monitored by circular dichroism at 277 nm. Circles represent the raw data recalculated to the fraction of unfolded DNA. Continuous lines represent the data fits used to calculate the melting temperatures displayed in the table.
Fig. S3. Analysis of cellular fatty acid composition. Strains were complemented with pHERD20T::accB (+accB), pHERD20T::accBC (+accBC) or carried the empty vector (+e.v.) as control. Strains were grown on LB agar plates with 0.2% arabinose and 400 µg/ml carbenicillin. Bars represent mean values ± SD from three independent experiments, *P < 0.1, ns = not significant according to one-tailed Mann-Whitney test compared to control strains with empty vector.
Fig. S4. Colony morphologies of complemented strains. SCV20265 and WTaroQ1accBC were complemented with pHerd20T::accB, pHerd20T::accBC or carried the empty vector (e.v.) as control. Representative photographs were taken from colonies grown for 7 days at room temperature on LB agar supplemented with Congo Red dye and 0.2% arabinose for the induced overexpression of accB or accBC.
**Fig. S5.** Intracellular c-di-GMP concentrations for revertant 1. Intracellular c-di-GMP concentrations were measured after planktonic cultivation for 24 hours in LB with 300 mM NaCl at 37°C. Bars represent mean values ± SD from three independent biological replicas, ***$P < 0.001$*** according to two-tailed Mann-Whitney test.

**Fig. S6.** Fatty acid composition under different growth conditions. Comparison of the amount of the three major fatty acids unsaturated palmitoleic acid (C16:1ω7), saturated palmitic acid (C16:0) and cis-vaccenic acid (C18:1ω7) of the total lipid fraction for SCV20265 (SCV) and
revertant 1 (Rev1) grown in planktonic cultures (black bars) or on agar plates (gray bars) with (A) 120 mM, (B) 300 mM or (C) without sodium chloride (NaCl). Bars represent mean values ± SD from three independent experiments, *P < 0.05, ns = not significant according to one-tailed Mann-Whitney test compared to planktonic conditions.
Fig. S7. Colony morphologies on Columbia blood agar plates. Colonies formed by wild-type strains PA14 and WT20265 and their respective mutant strains. All photographs were taken after growth at 37°C for 18 hours as representative examples.
Table S1. Strains and plasmids.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Reference</th>
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<tr>
<td><strong>Escherichia coli</strong></td>
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<td></td>
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<tr>
<td><em>E. coli</em> DH5α</td>
<td>F&lt;sup&gt;−&lt;/sup&gt; endA&lt;sup&gt;1&lt;/sup&gt;, glnV44, thi-1, recA&lt;sup&gt;1&lt;/sup&gt;, relA&lt;sup&gt;1&lt;/sup&gt;,</td>
<td>(46)</td>
</tr>
<tr>
<td></td>
<td>gyrA96, deoR, nupG, Φ80dlacZΔM15, Δ(lacZYA-argF)U169, hsdR17&lt;sup&gt;(rK&lt;sup&gt;−&lt;/sup&gt;mK&lt;sup&gt;+&lt;/sup&gt;)&lt;/sup&gt;, λ−</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> S17-1</td>
<td>C600::RP-4 2-(Tc::Mu) (Kn::Tn7) thi, pro, hsdR, hsdM+recA</td>
<td>(47)</td>
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<tr>
<td><em>E. coli</em> WM3064</td>
<td>thrB1004 pro thi rpsL hsdS lacZΔM15RP4-1360 Δ(araBAD)567 ΔdapA1341::[erm</td>
<td>W Metcalf, University of Illinois, Urbana</td>
</tr>
<tr>
<td></td>
<td>pir&lt;sup&gt;(wt)&lt;/sup&gt;]</td>
<td></td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCV20265</td>
<td>Clinical isolate</td>
<td>(12)</td>
</tr>
<tr>
<td>WT20265</td>
<td>Clinical isolate</td>
<td>(12)</td>
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<tr>
<td>Revertant 1-10</td>
<td><em>In vitro</em> generated from SCV20265</td>
<td>This study</td>
</tr>
<tr>
<td>PA14</td>
<td>wild-type</td>
<td>(48)</td>
</tr>
<tr>
<td>PA14&lt;sup&gt;aroQ1accBC&lt;/sup&gt;&lt;sub&gt;SCV&lt;/sub&gt;</td>
<td>PA14 wild-type with <em>aroQ1accBC</em> from SCV20265</td>
<td>This study</td>
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<tr>
<td>PA14&lt;sup&gt;wspF&lt;/sup&gt;&lt;sub&gt;SCV&lt;/sub&gt;</td>
<td>PA14 wild-type with <em>wspF</em> from SCV20265</td>
<td>This study</td>
</tr>
<tr>
<td>PA14&lt;sup&gt;aroQ1accBC&lt;/sup&gt;&lt;sub&gt;SCV&lt;/sub&gt;&lt;sup&gt;wspF&lt;/sup&gt;&lt;sub&gt;SCV&lt;/sub&gt;</td>
<td>PA14&lt;sup&gt;aroQ1accBC&lt;/sup&gt;&lt;sub&gt;SCV&lt;/sub&gt; with <em>wspF</em> from SCV20265</td>
<td>This study</td>
</tr>
<tr>
<td>PA14Δ<em>wspR</em></td>
<td>PA14 wild-type with in frame <em>wspR</em> deletion</td>
<td>This study</td>
</tr>
<tr>
<td>WT&lt;sup&gt;*aroQ1accBC&lt;/sup&gt;&lt;sub&gt;SCV&lt;/sub&gt;</td>
<td>WT20265 with <em>aroQ1-accBC</em> from SCV20265</td>
<td>This study</td>
</tr>
<tr>
<td>WT&lt;sup&gt;*aroQ1accBC&lt;/sup&gt;&lt;sub&gt;SCV&lt;/sub&gt;&lt;sup&gt;wspF&lt;/sup&gt;&lt;sub&gt;SCV&lt;/sub&gt;</td>
<td>WT&lt;sup&gt;*aroQ1accBC&lt;/sup&gt;&lt;sub&gt;SCV&lt;/sub&gt; with <em>wspF</em> from SCV20265</td>
<td>This study</td>
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<tr>
<td>SCV&lt;sup&gt;wspF&lt;/sup&gt;&lt;sub&gt;WT&lt;/sub&gt;</td>
<td>SCV20265 with <em>wspF</em> from WT20265</td>
<td>This study</td>
</tr>
<tr>
<td>Plasmid</td>
<td>Description</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>WT\textsubscript{wspF\textsubscript{SCV}}</td>
<td>WT20265 with \textit{wspF} from SCV20265</td>
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<td>WT\Delta\textit{wspR}</td>
<td>WT20265 with in frame \textit{wspR} deletion</td>
<td>This study</td>
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<tr>
<td>SCV\Delta\textit{wspR}</td>
<td>SCV20265 with in frame \textit{wspR} deletion</td>
<td>This study</td>
</tr>
<tr>
<td>Rev1\Delta\textit{wspR}</td>
<td>Revertant 1 with in frame \textit{wspR} deletion</td>
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<tr>
<td>pEX18Ap</td>
<td>Gene replacement vector, \textit{Amp}\textsuperscript{R}, \textit{oriT}\textsuperscript{+}, \textit{sacB}\textsuperscript{+}</td>
<td>(35)</td>
</tr>
<tr>
<td>pEX18Ap::\textit{aroQ1\textsubscript{SCV}}</td>
<td>pEX18Ap with \textit{aroQ1accBC} from the SCV20265 as \textit{EcoRI/HindIII} fragment</td>
<td>This study</td>
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<td>pEX18Ap::\textit{wspF\textsubscript{SCV}}</td>
<td>pEX18Ap with \textit{wspF} from the WT20265 as \textit{EcoRI/HindIII} fragment</td>
<td>This study</td>
</tr>
<tr>
<td>pEX18Ap::\textit{wspF\textsubscript{WT}}</td>
<td>pEX18Ap with \textit{wspF} from the WT20265 as \textit{EcoRI/HindIII} fragment</td>
<td>This study</td>
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<tr>
<td>pEX18Ap::\textit{wspR_KO}</td>
<td>pEX18Ap with \textit{wspR} knockout allele from WT20265 as \textit{EcoRI/HindIII} fragment</td>
<td>This study</td>
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<tr>
<td>pHERD20T</td>
<td>Shuttle vector, inducible with arabinose, \textit{Amp}\textsuperscript{R}</td>
<td>(37)</td>
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<td>pHERD20T::\textit{accB}</td>
<td>\textit{accB} gene cloned into MCS of pHERD via \textit{EcoRI/HindIII}</td>
<td>This study</td>
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<tr>
<td>pHERD20T::\textit{accBC}</td>
<td>\textit{accBC} gene cloned into MCS of pHERD via \textit{EcoRI/XbaI}</td>
<td>This study</td>
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<td>pUCP20</td>
<td>Shuttle vector, \textit{Amp}\textsuperscript{R}</td>
<td>(49)</td>
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<td>pUCP20::\textit{accB_TOE\textsubscript{SCV}}</td>
<td>\textit{accB} 5'-UTR (-173 to +60) of SCV20265 flanked by a T7 promoter and an \textit{EcoRV} site subcloned into \textit{SmaI} of pUCP20</td>
<td>This study</td>
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pUCP20::accB_TOE<sub>Rev1</sub>

and an EcoRV site subcloned into 
SmaI of pUCP20

This study

pUCP20::accB_TOE<sub>Rev4</sub>

accB 5′-UTR (-173 to +60) of 
revertant 4 flanked by a T7 promoter 
and an EcoRV site subcloned into 
SmaI of pUCP20

This study

pUCP20::aroQ1-accB<sub>WT</sub>-His<sub>6</sub>

pUCP20 with <i>aroQ1accB</i> from 
WT20265 with C terminal His<sub>6</sub>-tag as 
EcoRI/BamHI fragment

This study

pUCP20::aroQ1-accB<sub>SCV</sub>-His<sub>6</sub>

pUCP20 with <i>aroQ1accB</i> from 
SCV20265 with C terminal His<sub>6</sub>-tag as 
EcoRI/BamHI fragment

This study

pUCP20::aroQ1-accB<sub>Rev</sub>-His<sub>6</sub>

pUCP20 with <i>aroQ1accB</i> from 
Revertant 1 with C terminal His<sub>6</sub>-tag as 
EcoRI/BamHI fragment

This study
<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
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<tr>
<td>accB_TOE_fw1</td>
<td>GAAATTAATACGACTCACTATAGGAAGTGCACTGTGTCG</td>
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<tr>
<td>accB_TOE_rev1</td>
<td>TAAGATATCCTCGTGATACCAGGACTC</td>
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<td>accB_TOE_R-10</td>
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<td>aroQ_rev</td>
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<td>TAAGATATCCTCGTGATACCAGGACTC</td>
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<td>wspR_KO_Up_fw1</td>
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<tr>
<td><strong>For qRT-PCR</strong></td>
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</tr>
<tr>
<td>rpsL TM</td>
<td>6FAM-AGGTTTGTGACCTTCACCACCAGGATGT-BBQ*</td>
</tr>
<tr>
<td>accB TM</td>
<td>6FAM-ACGCTCTGCGAGCTGCGAGA-BBQ*</td>
</tr>
<tr>
<td>accC TM</td>
<td>6FAM-TTGAATCAGCT CGAGCTGAGAGTCAGCGA-CC-BBQ*</td>
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**rpsL S**  
TGCGGTGTACGTCTGACCAA

**rpsL R**  
GCGCAGGGTGTGGTAAC

**aceB S**  
CTGAACGGCAACGTGGTT

**aceB R**  
GCTTCGACGATGCACAGGA

**aceC F**  
GGTCGGCTACCCGGTGA

**aceC A**  
GTTGGTCAGGAACTTCTCCAGG

*6FAM represents 6-carboxyfluorescein and BBQ stands for BlackBerry Quencher for the TaqMan (TM) Probes*
Table S3. DNA oligonucleotides used for CD spectroscopy.

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<td>WT</td>
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<td>SCV</td>
<td>CAACTCCCCTGACCTCACTGGGAGTGC</td>
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<tr>
<td>Rev1</td>
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<td>Rev6</td>
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</tr>
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<td>Rev8</td>
<td>CAACTCCCCTGACCTCACTAGGAGTGC</td>
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