Additional file 4:

The experimental data to verify the mutants and the strains with gene overexpression

5.1 Verification of the mutants by genomic PCR.

5.1.1 Verification of the mutant strain GP16 (gltP mutant)

The gltP mutant [168Δ(gltP::cam')] was constructed by insertion of chloramphenicol resistance gene (cam' or cat) in gltP wild-type. The upstream region of gltP (UgltP) was amplified by F-5UgltP-SalI and R-5UgltP-KpnI and the downstream region of gltP (DgltP) was amplified by F-3DgltP-SacI and R-3DgltP-EcoRI. The cat gene was amplified by F-cat-KpnI and R-cat-SacI. All three PCR products (UgltP, DgltP, and cat gene) were digested by the indicated restriction enzymes and cloned into pUC119 as shown in following figure.

The constructed vector was transformed into B. subtilis 168 (WT). The transformants were selected as chloramphenicol resistance clones. The genomic of GP16 (ΔgltP::cam') strain was verified by genomic PCR. The pair of primer F-5UgltP-SalI and R-3DgltP-EcoRI (highlighted in yellow) was used for PCR amplification resulting in a 2.8-kb PCR product of mutants and a 3.1-kb PCR product of WT as shown in the following figure. The clone #GP16 was selected for further study.
5.1.2 Verification of the mutant strain B934 (proB mutant)

The proB mutant 168Δ(proB::spc) was constructed by insertion of spectinomycin resistance gene (spc' or spc) in proB wild-type. The covered region of proB was amplified by F-53proB and R-53proB-SphI. The spc gene was amplified by F-spc-SacII and R-spc-SacII resulting a 1.05-kb PCR product. The wild-type proB gene was cloned into pUC119 at EcoRI-SphI site, and then the spc gene was flanked into the vector at SacII sites. The construction procedure is shown in the following figure.

The constructed vector was transformed into B. subtilis 168 (WT). The transformants were selected as spectinomycin resistance clones. The mutant strain B934 (AprOB::spc') strain was verified by genomic PCR. The pair of F-53proB and R-53proB-SphI (highlighted in yellow) was used for PCR amplification resulting in a 2.8-kb PCR product of mutants and
a 2.3-kb PCR product of WT as shown in the following figure. The mutant B934 was used for further experiment, and for the construction of the mutant strain BH901.

The genomic PCR verification of proB mutants. M: GeneRuler 1 kb DNA Ladder (Thermo Scientific); WT: B. subtilis 168; B934: 168ΔproB::spc

5.1.3 Verification of the mutant strain BH901 (proHJ mutant)

The proHJ mutant 168Δ(proB::spc') Δ(proHJ::cam') or B934Δ(proHJ::cam') were constructed by insertion of chloramphenicol resistance gene (cam' or cam) in proHJ wild-type. The covered region of proHJ was amplified by F-53proHJ-BamHI and R-53proHJ-KpnI. The cat gene was amplified by F-cat-XhoI and R-cat-BglII resulting a 0.8-kb PCR product. The wild-type proHJ gene was cloned into pUC119 at BamHI-KpnI site. Then the cat gene was flanked into the vector at XhoI-BglII site. The construction procedure is shown in the following figure
The constructed vector was transformed into \textit{B. subtilis} 168 (WT) and the mutant strain B934. The transformants were then selected as chloramphenicol resistance clones. BH901 mutant strains (\textit{\Delta proB::spc'}, \textit{\Delta proHJ::cam'}) and H972 (\textit{\Delta proHJ::cam'}) were verified by genomic PCR. The pair of F-53proHJ-BamHI and R-cat-BglII (highlighted in yellow) was used for PCR amplification resulting in a 1.8-kb PCR product as shown in the following figure.

The genomic PCR verification of the \textit{proHJ} mutants. M: GeneRuler 1 kb DNA Ladder (Thermo Scientific); WT: \textit{B. subtilis} 168; BH901: \textit{\Delta proB::spc'}, \textit{\Delta proHJ::cam'}; H972: \textit{\Delta proHJ::cam'}. 
5.2 Verification of the strains with gene overexpression (HK-GOX and HK’-HJOX) using qRT-PCR.

The strains with the overexpressed gene (HK-GOX: a recombinant strain with an overexpressed GltP under P43 promoter; and HK’-HJOX: a recombinant strain with an overexpressed ProHJ under its original promoter) were verified using qRT-PCR of the gene target. The strains were cultivated in LB medium to early-exponential phase, then further incubated for 1 h, and extracted the RNA. qRT-PCR analysis was carried out using the specific primers indicated in Additional file 3. The level of gene-expression was then compared to that of the HK strain control (168 carrying pHK empty-vector), and expressed as expression fold change (as shown below). The results are mean of two independent biological replicates.

<table>
<thead>
<tr>
<th>Strain</th>
<th>A target gene</th>
<th>Expression fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>HK-GPOX</td>
<td><em>gltP</em></td>
<td>170.1 ± 39.8</td>
</tr>
<tr>
<td>HK’-HJOX</td>
<td><em>proH</em></td>
<td>371.7 ± 23.9</td>
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