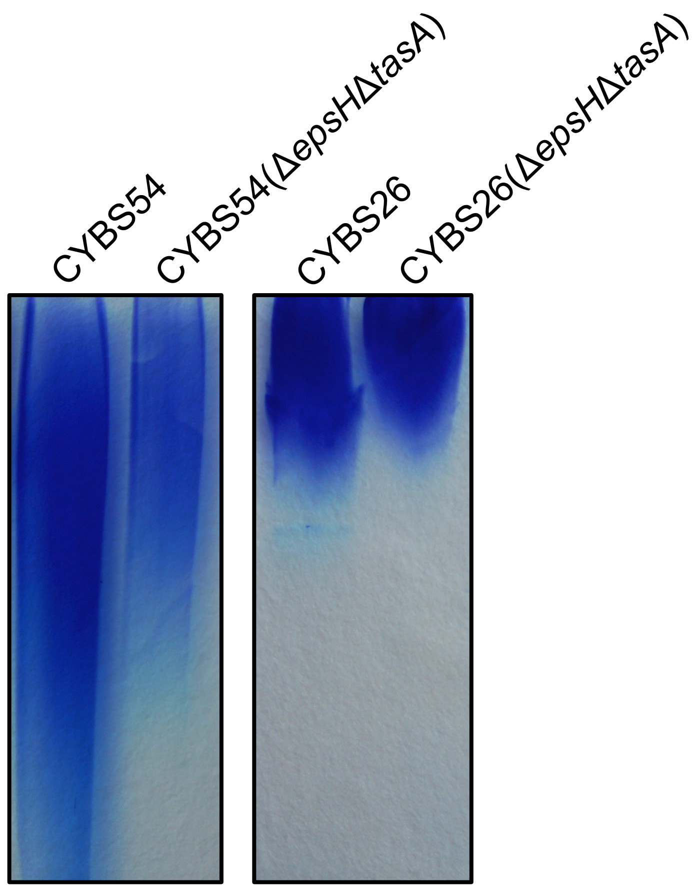

Supplemental Figure 1

```
3610      GATTGATTTGAAATGCATATTAAATACAAACAGCAGGGTGTGACTATACGTCCAGAAGGATATCAGGAGAAAAAT
SM21      GATTGATTTGAAATGCATATTAAACACAAACACCAGGGTGTGACTATACGTCCAGAAGGATATCAGGGGAAAAAT
CYBS9     GATTGATTTGAAATGCATATTAAACACAAACACCAGGGTGTGACTATACGTCCAGAAGGATATCAGGGGAAAAAT
CYBS14    GATTGATTTGAAATGCATATTAAATACAAACAGCAGGGTGTGACTATACGTCCAGAAGGATATCAGGAGAAAAAT
CYBS26    GATTGATTTGAAATGCATATTAAATACAAACAGCAGGGTGTGACTATACGTCCAGAAGGATATCAGGAGAAAAAT
CYBS54    GATTGATTTGAAATGCATATTAAATACAAACAGCAAGGTGTGACTATACGTCCAGAAGGATATCAGGAGAAAAAT
          ************************.*******.**:********************************.******

             CcpA
          <---------->
3610      GAAAGCGCATTCTCTTTGATTTTTTTAAAAAGTGATAAGGTATAAGTCCTGCTGTTTTCCAAAAATAGAAAACAG
SM21      GAAAGCGCATTCTCTTCGATTTTTAAAAAAAGTGATGAGGTATAAGTCCTGCTGTTTTCCAAAAATAGAAAACAG
CYBS9     GAAAGCGCATTCTCTTCGATTTTTAAAAAAAGTGATGAGGTATAAGTCCTGCTGTTTTCCAAAAATAGAAAACAG
CYBS14    GAAAGCGCATTCTCTTCGATTTTTAAAAAAAGTGATAAGGTGTAAGTCCTGCTGTTTTCCAAAAATAGAAAACAG
CYBS26    GAAAGCGCATTCTCTTCGATTTTTAAAAAAAGTGATAAGGTGTAAGTCCTGCTGTTTTCCAAAAATAGAAAACAG
CYBS54    GAAAGCGCATTCTCTTTGATTTTTAAAAAAAGTGATAAGGTGTAAGTCCTGCTGTTTTCCAAAAATAGAAAACAG
          ****************.*******::**********.****.*********************************

                                                                                DegU
                                                                           <--------
3610      TTTGTAGGTATAAAATCTCTTTCAAAAGAGAAGTTTGGCTTAGTCGATTAGGGAAGATTATGTTACATAATGCCG
SM21      TTTGTAGGTATAAAATCTCTTTCAAAAGAGAAGTTTGGCTTAGTCGATTAGGGAAGATTATGTTACATAATGCCG
CYBS9     TTTGTAGGTATAAAATCTCTTTCAAAAGAGAAGTTTGGCTTAGTCGATTAGGGAAGATTATGTTACATAATGCCG
CYBS14    TTTGTAGGTATAAAATCTCTTTCAAAAGAGAAGTTTGGCTTAGTCGATTAGGGAAGATTATGTTACATAATGCCG
CYBS26    TTTGTAGGTATAAAATCTCTTTCAAAAGAGAAGTTTGGCTTAGTCGATTAGGGAAGATTATGTTACATAATGCCG
CYBS54    TTTGTAGGTATAAAATCTCTTTCAAAAGAGAAGTTTGGCTTAGTCGATTAGGGAAGATTATGTTACATAATGCCG
          ***************************************************************************

          --->-35                      -10        +1
3610      ATTGAGAATTCATAGTGATTCTATATACTGATGAATGAATTTACAACAATATAGAAGGAGATGTCGAAAAGCA
SM21      ATTGAGAATTCATAGTGATTCTATATACTGATGAATGAATTTACAACAATATAGAAGGAGATGTCGAAAAGCA
CYBS9     ATTGAGAATTCATAGTGATTCTATATACTGATGAATGAATTTACAACAATATAGAAGGAGATGTCGAAAAGCA
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CYBS26    ATTGAGAATTCATAGTGATTCTATATACTGATGAATGAATTTACAACAATATAGAAGGAGATGTCGAAAAGCA
CYBS54    ATTGAGAATTCATAGTGATTCTATATACTGATGAATGAATTTACAACAATATAGAAGGAGATGTCGAAAAGCA
          *********************************************.**:************************
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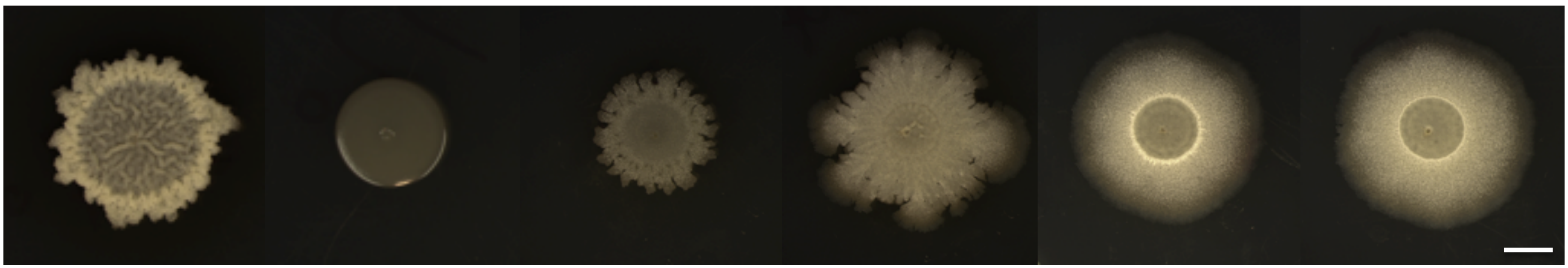
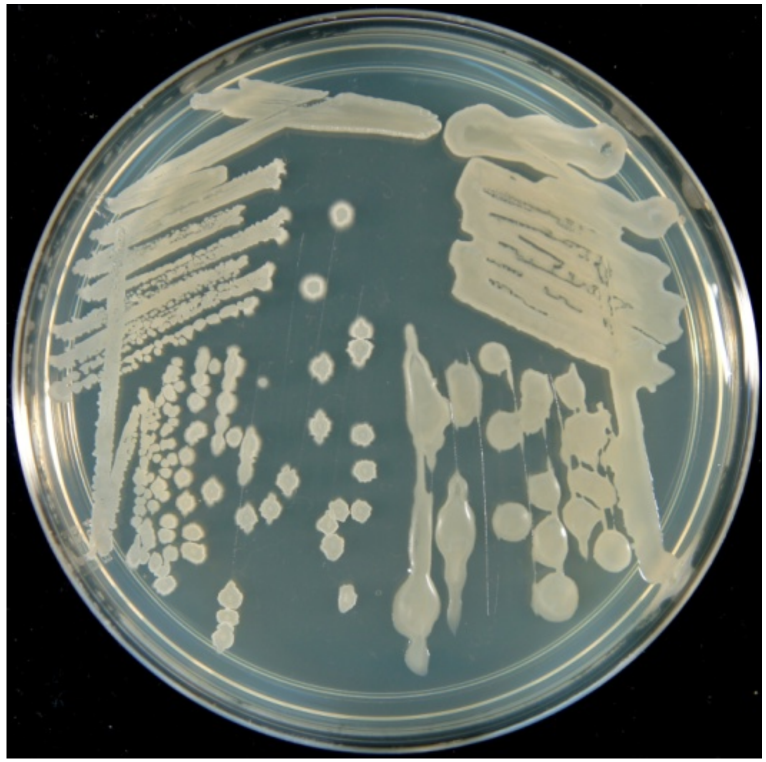
Supplemental Figure 2

3610 | Δ*epsH* | Δ*tasA* | Δ*pgs* | Δ*tasA* + Δ*pgs* | Δ*epsH* + Δ*pgs*



Supplemental Figure 3



3610 Δ*motA*

Supplemental Figure 4

Supplemental figure legends

Fig. S1. SDS-PAGE for methylene blue stained samples containing purified γ-PGA from the environmental isolates CYBS54, CYBS26 and their corresponding Δ*epsH*Δ*tasA* double mutants. Purification of PGA was described in the Methods.

Fig. S2. DNA sequence alignment of the *pgs* promoter regions from various *B. subtilis* environmental strains. The *pgs* promoter sequences were obtained by PCR amplification of the corresponding regions using genomic DNAs from selected *B. subtilis* environmental strains and primers P_{pgsA}-F1 and P_{pgsA}-R1 (Table S1) and by DNA sequencing. The DNA sequence alignment was performed by using the program ClustalW (http://www.genome.jp/tools/clustalw/). The columns with nucleotide polymorphism are highlighted in yellow and green. Yellow represents consensus while green indicates changes. The -10 and -35 motifs of the sigma A-dependent promoter, and the transcription start (+1) were experimentally confirmed and are indicated here (1). A CcpA binding site (*cre* box) was also experimentally characterized in a previous study (2)and indicated here.

Fig. S3. Biofilm complementation by various matrix mutants. Shown here are colony biofilms on LBGM agar plates formed by the wild type(3610), the Δ*epsH* mutant (RL3852), the Δ*tasA* mutant(SB505), the Δ*pgs* mutant(FY6), a 1:1 mixture of the Δ*tasA* and Δ*pgs* mutants, and a 1:1 mixture of Δ*epsH* and Δ*pgs* mutants. 2-ul log phase cells were spotted onto the center of the plates and incubated at 37°C for 2 days before images were taken. The scale bar represents 5 mm in length.

Fig. S4. The Δ*motA* mutant shows a colony mucoidy phenotype. The wild type strain (3610) and the Δ*motA* mutant in 3610 (CY258) were streaked out on the LB agar plate and incubated for 16 hours at 37°C before the picture was taken.

Supplemental references

1. **Ohsawa T, Tsukahara K, Ogura M.** 2009. *Bacillus subtilis* Response Regulator DegU Is a Direct Activator of *pgsB* Transcription Involved in γ-Poly-glutamic Acid Synthesis. Bioscience, Biotechnology, and Biochemistry **73:**2096-2102.

2. **Ishii H, Tanaka T, Ogura M.** 2013. The *Bacillus subtilis* Response Regulator Gene degU Is Positively Regulated by CcpA and by Catabolite-Repressed Synthesis of ClpC. Journal of Bacteriology **195:**193-201.

Table S1. Oligonucleotides used in this study.

Primer name	**Primer sequence (5'-3')**
pdgS-P1	AAGATACGCTTGGAGAATTTGCGAAGCAAA
pdgS-P2	CAATTCGCCCTATAGTGAGTCGTTTTTATTATCTCCTCCTC
pdgS-P3	CCAGCTTTTGTTCCCTTTAGTGAGAAGTATACGGGTGCAATA
pdgS-P4	CGATTTAGGTGTATAATGAACGGGATGGCG
pgs-P1	CGCCGTTAAATCGGTCTTGAGCG
pgs-P2	CAATTCGCCCTATAGTGAGTCGTGTATGACAGCACAGGCTA
pgs-P3	CCAGCTTTTGTTCCCTTTAGTGAGAAGTATACGGGTGCAATA
pgs-P4	CGATTTAGGTGTATAATGAACGGGATGGCG
PpgsB-F1	GTACAAGCTTATGACCTTGTCCTTAAGAAACAG
PpgsB-R1	GTACGGATCCCTTCTATATTGTTGTAAATTC