

Supplementing information for:

The GABA permease GabP serves as the third proline transporter of *Bacillus subtilis*

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Running head: GabP-mediated proline uptake

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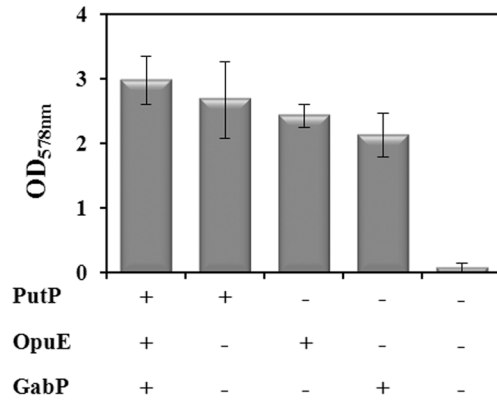


FIG S1 The growth of the *B. subtilis* wild-type strain and its mutant derivatives pre-exposed to proline in a minimal medium containing only proline as the carbon source. Cells of the *B. subtilis* wild-type strain JH642 and its mutant derivatives ACB275 (*opuE gabP*), ACB277 (*putP gabP*), SMB12 (*putP opuE*), and ACB279 (*putP opuE gabP*) were pre-grown in SMM that contained 14 mM glucose and 32 mM proline. The cells were then carefully washed two times and aliquots were used to inoculate SMM containing only proline (32 mM) as the sole carbon and energy source. The cells were inoculated to an OD₅₇₈ of 0.2 and the growth yields of the cultures were determined by measuring the OD₅₇₈ after 16 h of incubation in shaking water bath set at 37 °C. The values given represent the means of two independently grown cultures, and the error bars indicate standard deviations.

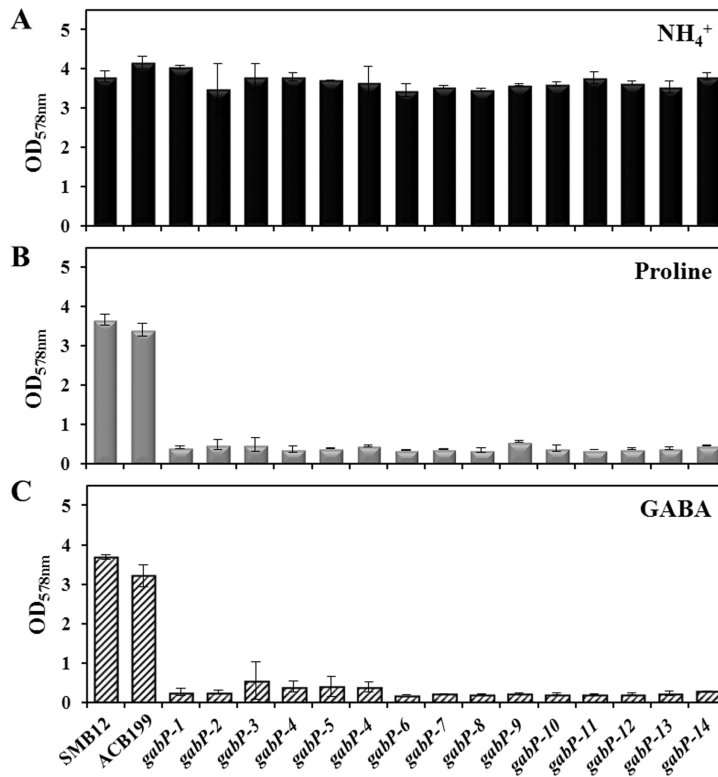


FIG S2. Point mutations in GabP abolish growth of the *B. subtilis* mutant strain (*putP opuE*) in minimal medium with proline or GABA as nitrogen sources. Cultures of SMB12 (*putP opuE*), ACB199 (*putP opuE ywcA yodF*), and 15 DHP-resistant mutant strains (*gabP-1* to *gabP-14*) (Table 2) were used to inoculate SMM with glucose as the carbon source and either ammonium (A), proline (B) or GABA (C) as the nitrogen source (final concentration of ammonium, proline and GABA was 30 mM). The growth yield of the cultures was measured after 16 h of incubation at 37 °C. The values represent the means of two independently grown cultures, and the error bars indicate standard deviations.

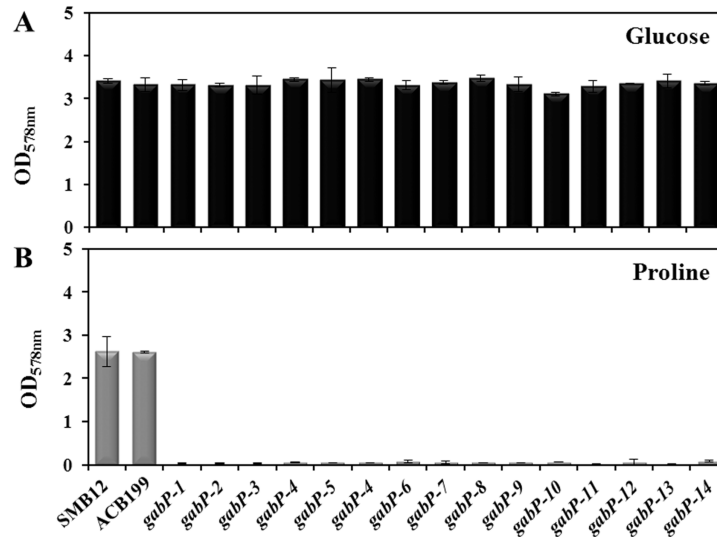


FIG S3. Point mutations in GabP abolish the ability of the *B. subtilis* mutant strain (*putP opuE*) to grow on proline as the sole carbon source. Cultures of SMB12 (*putP opuE*), ACB199 (*putP opuE ywcA yodF*) and 15 DHP-resistant mutant strains (*gabP-1* to *gabP-14*) (Table 3) were used to inoculate SMM with ammonium as the nitrogen source and either glucose (A) or proline (B) as the carbon source (final concentration of glucose and proline was 28 mM and 33 mM, respectively). The growth yield of the cultures was measured after 16 h of incubation at 37 °C. The values represent the means of two independently grown cultures, and the error bars indicate standard deviations.