Supplemental material:

High puritay hydroxyectoine production based on the

***Pseudomonas stutzeri ectABCD/ask* genecluster**

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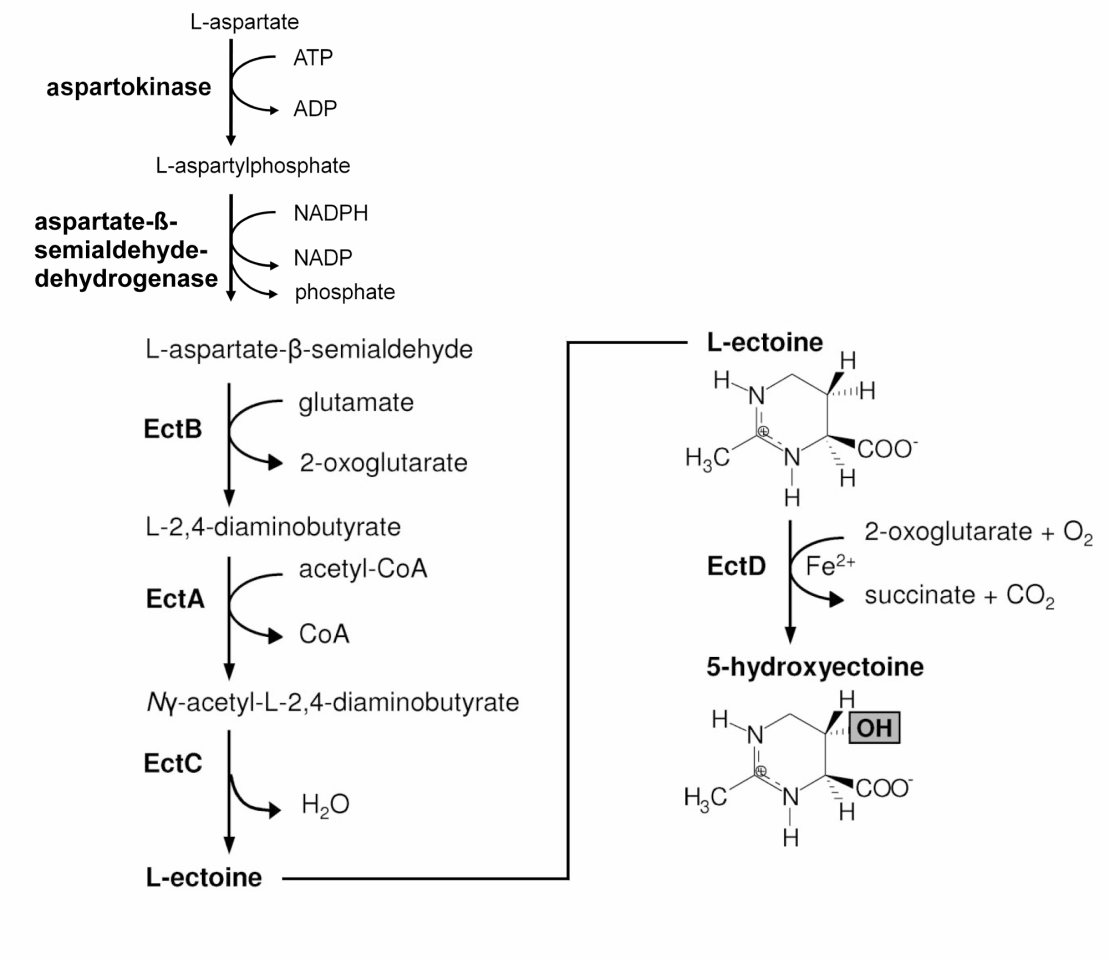
Figure S01 – Hydroxyectoine biosynthesis pathway



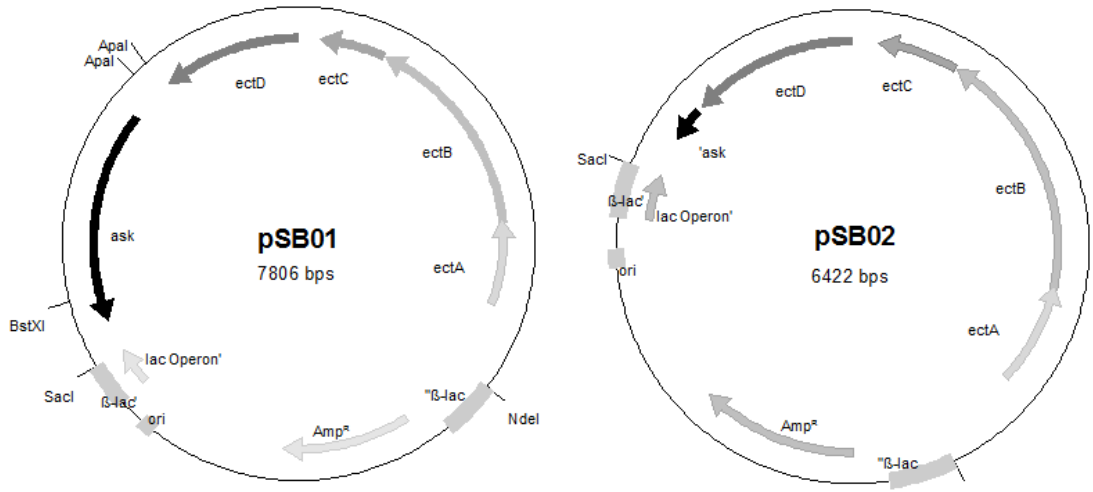
(Hydroxy-) Ectoine biosynthesis branches from biosynthesis of the aspartate family amino acids: Aspartylphosphate is converted into ectoine in three steps. This is followed by hydroxylation to hydroxyectoine.

See also :

Bestvater, T., P. Louis and E. A. Galinski. 2008. *Heterologous ectoine production in Escherichia coli: by-passing the metabolic bottle-neck*. Saline Systems. **4**:12.

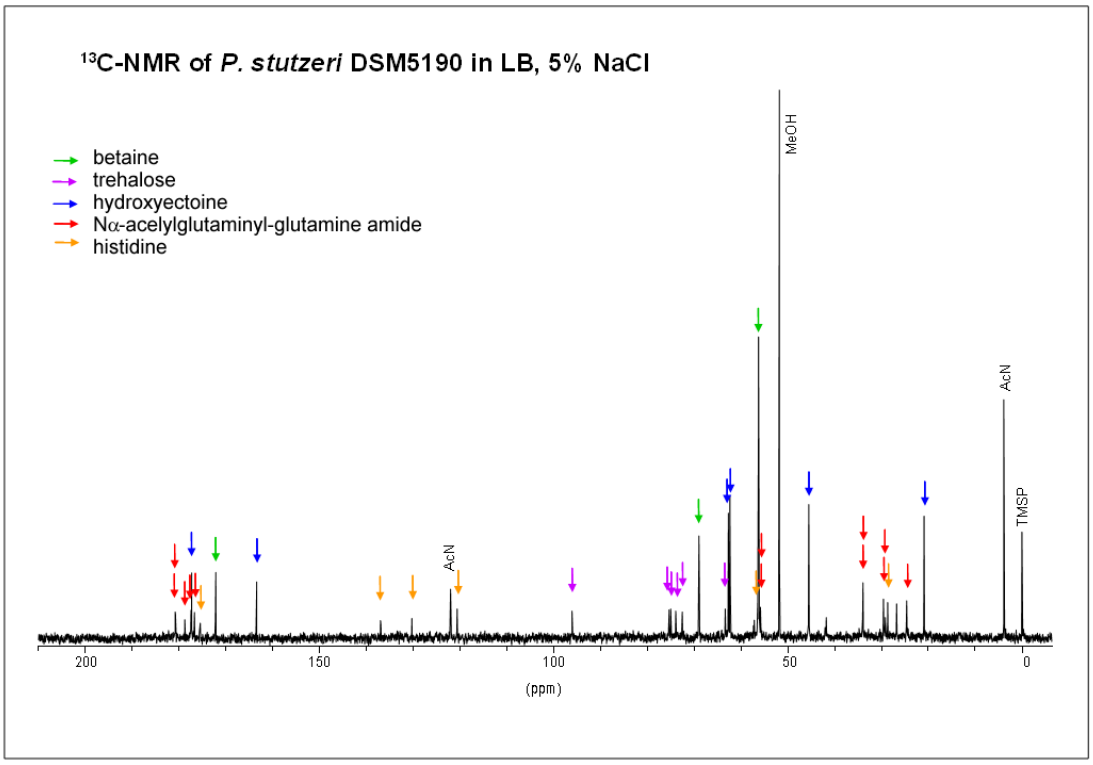
Bursy, J., A. U. Kuhlmann, M. Pittelkow, H. Hartmann, M. Jebbar, A. J. Pierik and E. Bremer. 2008. *Synthesis and uptake of the compatible solutes ectoine and 5-hydroxyectoine by Streptomyces coelicolor A3(2) in response to salt and heat stresses*. Appl Environ Microbiol. **74**:7286-7296.

Figure S02 – Vector maps pSB01 and pSB02



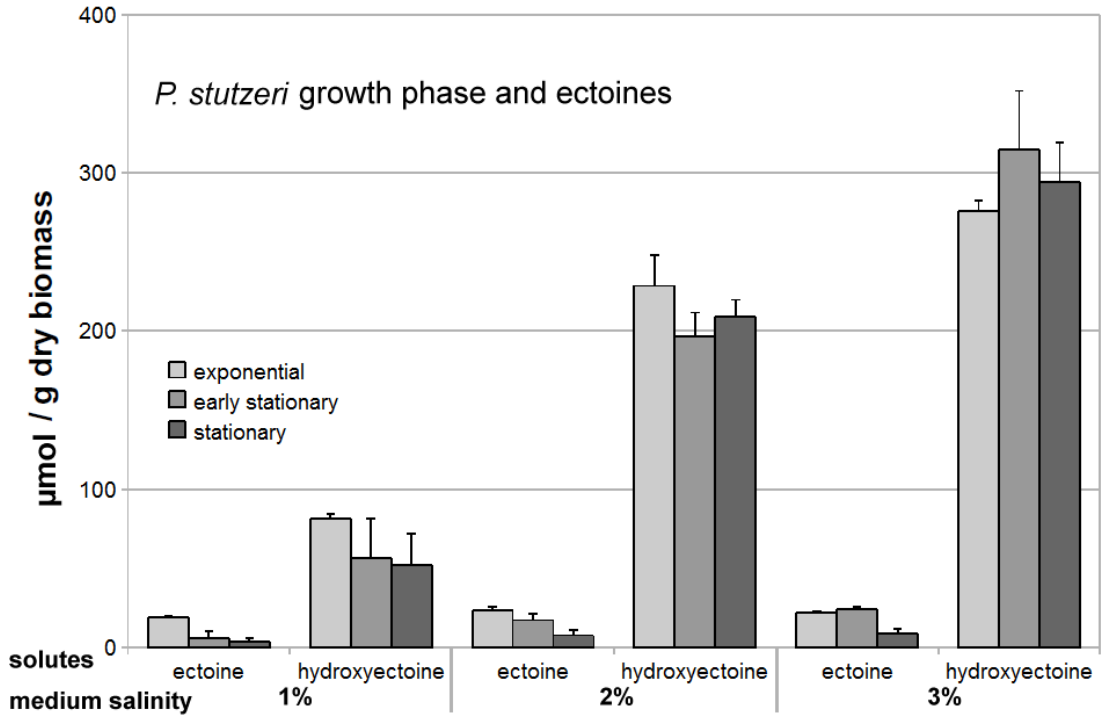
The pUC18 based vector pSB01 carries the complete hydroxyectoine biosynthesis gene cluster including the genes for transacetylase *ectA*, transaminase *ectB*, ectoine synthase *ectC*, ectoine hydroxylase *ectD* and an aspartokinase *ask*. The genes are oriented in opposite direction with respect to the *lac*-promoter and thus only under control of the native *P. stutzeri* promoters. In pSB02 the major part of *ask*, including the active site, is deleted via the *Apa*I and *Bst*XI restriction sites. The ampicillin resistance gene amp^R, origin of replication *ori* and remainders of the β-lactamase gene from pUC18 are also marked.

Figure S03 – ^{13}C-NMR-spectrum of a *Pseudomonas stutzeri* solute extract



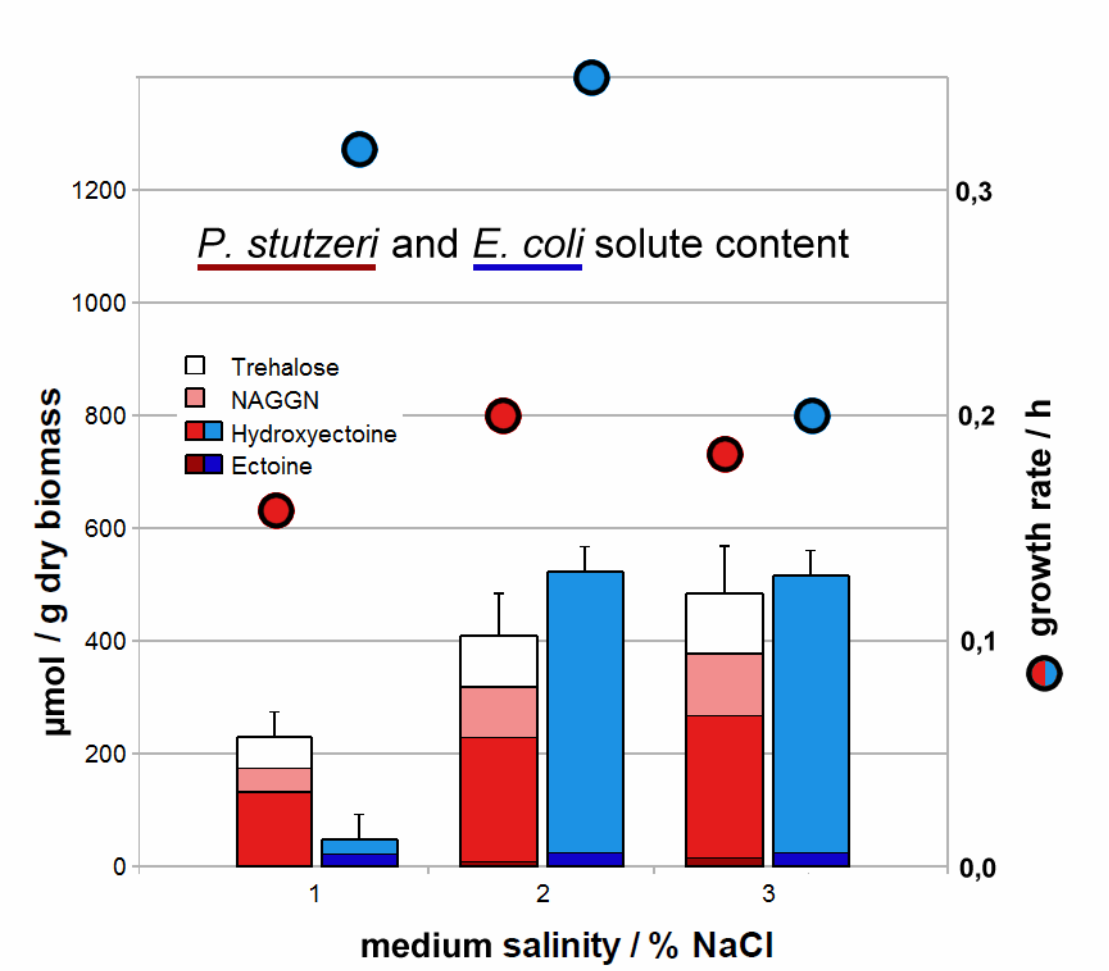
P. stutzeri DSM 5190^{T} was grown in Luria Bertani (LB) medium at 5% salinity and extracted as described. A ^{13}C-NMR spectrum was recorded from 35 mg raw extract in D_2O. Reference signals are trimethylsilyl-propionic acid (TMSP), acetonitrile (AcN) and methanol (MeOH). Signals for betaine (green), trehalose (magenta), hydroxyectoine (blue), NAGGN (red) and histidine (orange) were allocated by comparison with spectra of pure substances.

Figure S04 – Production of ectoines in *P. stutzeri* is growth phase independent



P. stutzeri DSM 5190^{T} was grown in MM63 medium at different salinities. Samples were taken in different growth phases and extracted as described. Within experimental errors ectoine content decreases slightly but not significantly from exponential via early stationary to stationary growth phase. Hydroxyectoine content stays at approximately the same level.

Figure S05 – Comparing solute production in *E. coli* and *P. stutzeri*



Main solutes synthesized *de novo* in MM63 by *P. stutzeri* DSM5190^{T} (red shades) and *E. coli* DH5α pSB01 (blue shades) are trehalose (white, only *Pseudomonas*), NAGGN (light colour, *Pseudomonas*) and hydroxyectoine (medium colour). Ectoine (dark colour) is present only in minor amounts. Concentration of hydroxyectoine is more than double in the *E. coli* mutant compared to the native producer, ectoine levels are approximately the same. Also, in *E. coli* there is no linear increase of solute production with salinity.