Ectoine Production from Soy Sauce Cake-Derived Medium by the Moderately Halophilic *Halomonas elongata*

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ABSTRACT

The moderately halophilic *Halomonas elongata* accumulates osmolytes such as ectoine (Ect) and glycine betaine (GB) under high-salinity conditions and is commercially utilized as cell factories for producing Ect, a high-value cosmetic and pharmaceutical ingredient. *H. elongata* OUT30018, maintained in our laboratory, is distinguished within the species by its ability to assimilate lignocellulose-derived polysaccharides and putrefactive amines as nutrient sources. To develop a novel upcycling technology for producing valuable chemicals from waste biomass, we here present a method to convert soy sauce cake, a high-salinity waste from the soy sauce industry, into a medium for culturing *H. elongata* OUT30018 for Ect production.

To convert soy sauce cake into a culture medium, we developed an alkaline hydrolysis method using NaOH to break down the cake's macromolecular components into forms readily usable by microorganisms. We compared the growth of *H. elongata* OUT30018 in soy sauce cake hydrolysate (SH) medium treated with 0%, 2.5%, 5%, and 10% (w/v) NaOH and found that the highest cell yield was achieved in the medium prepared with 5% (w/v) NaOH. Furthermore, *H. elongata* OUT30018 cells cultivated in 5% NaOH-treated SH medium containing 2.5 M NaCl accumulated 1.5 times as much Ect as those grown in LB medium. Interestingly, the amount of GB accumulated in the cells grown in the SH medium was approximately one-tenth of that in cells grown in LB medium. This is likely because SH medium contains less GB than LB medium. A high concentration of GB in the medium is known to suppress expression of the *ect* operon, which encodes enzymes in the Ect biosynthetic pathway. This finding demonstrates that SH medium is an excellent substrate for Ect production by *H. elongata* OUT30018.

Furthermore, we engineered more efficient cell factories for Ect production by generating *H. elongata* deletion mutants lacking the genes encoding enzymes involved in Ect catabolism and Ect hydroxylation. These mutants are *H. elongata* Δ*doeA*, lacking the *doeA* gene encoding Ect hydrolase for Ect assimilation; *H. elongata* Δ*ectD,* lacking the *ectD* gene encoding Ect hydroxylase for biosynthesis of 5-hydroxyectoine (HydEct); and *H. elongata* Δ*doeA*Δ*ectD*, a double-deletion mutant lacking both genes. When cultivated in SH medium, both *H. elongata* Δ*ectD* and Δ*doeA*Δ*ectD* showed higher Ect accumulation than the wild-type *H. elongata* OUT30018. These findings indicate that deletion of *ectD* gene further enhances Ect yield by suppression of HydEct production. Overall, our results indicate that *H. elongata* OUT30018 is an exceptionally powerful platform for upcycling high-salinity waste biomass into high-value chemicals.

Keywords: Soy sauce cake/ High salinity/ *Halomonas elongata*/ Ectoine/5-Hydroxyectione/ Glycine betaine