

Salt-tolerant mechanism of  $\gamma$ -glutamyltranspeptidase from *Bacillus subtilis*.

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If the substrate of the hydrolysis reaction of GGT is glutamine, the reaction is a “glutaminase” reaction. Soy sauce is a traditional Japanese seasoning and its umami taste depends mainly on the amount of glutamic acid. During its fermentation, soy proteins are digested into peptides by proteases from *Aspergillus oryzae* or *sojiae*, and then the peptides are cleaved into amino acids by their peptidases. Glutamine liberated is hydrolyzed to glutamic acid by glutaminase (Fig. 5). If glutaminase is insufficient, glutamine is converted spontaneously to tasteless or slightly sour pyroglutamic acid. Therefore, glutaminase is one of the most important enzymes for flavor enhancement in the manufacture of soy sauce. Soy sauce fermentation is performed in the presence of 18 % (~3 M) NaCl at pH 5.5 to prevent contamination. In the presence of such high concentration of NaCl, the activity of *Aspergillus* glutaminase is strongly inhibited. Therefore, salt-tolerant glutaminases were searched for in bacteria to apply to the fermentation mixture of soy sauce. We found that *Bacillus subtilis* synthesizes salt-tolerant GGTs. To elucidate the reason why *B. subtilis* GGT is salt-tolerant, salt-tolerant mutant of *Escherichia coli* GGT which is originally not salt-tolerant was isolated.

The large differences of *B. subtilis* GGT from *E. coli* GGT are the 14 amino acids insertion near the C-terminal of the large subunit and the 10 amino acids deletion at the center of the small subunit. The same insertion and deletion, and their combination were introduced in *E. coli* GGT. The activity of the deletion-type and the combination-type was severely reduced and the insertion-type was more salt-sensitive than the wild-type.

We developed an effective screening method employing the activity staining. Error-prone PCR was performed on the *E. coli ggt* gene and two salt-tolerant mutants were isolated using this screening method. We also found that the salt-tolerance depends on the substrates. The mutants we isolated were salt-tolerant using  $\gamma$ -glutamyl- $\alpha$ -naphthylamide as a substrate, but they were not salt-tolerant using glutamine.