Studies on Theanine Production by Coupled Fermentation with Energy Transfer

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The demand for theanine, which renders gracefully sweet taste of Japanese green tea, becomes great due to its favorable physiological functions. Although a practical theanine production is accomplished by using γ-glutamyl transfer reaction of bacterial enzyme, the method has several shortcomings. The objective of the present studies is establishing a new procedure of theanine production by use of an enzyme catalyzing similar reaction to that of theanine synthetase (glutamic acid (Glu) + ethylamine (EA) + ATP → theanine + ADP + inorganic phosphate (Pi)), in which ATP is regenerated by the sugar fermentation of baker's yeast designated as "the coupled fermentation with energy transfer."

In Chapter 1, an enzyme usable for theanine production was searched in methylamine- assimilators, and glutamine synthetase (GS: Glu + ammonia + ATP → Glutamine + ADP + Pi) of *Pseudomonas taetrolens* Y-30 was selected. The enzyme had certain theanine-forming activity under an appropriate condition.

Chapter 2 demonstrated that *P. taetrolens* Y-30 GS formed 170 mM theanine in an optimum reaction mixture of the coupled fermentation with energy transfer. The results indicated that (1) supply of large amounts of the GS or of a new enzyme with higher theanine-forming activity than *P. taetrolens* Y-30 GS and (2) control of ATP-consuming reaction(s) in yeast cells are necessary to increase the theanine. Analysis of the inhibitory effect of EA on the yeast sugar fermentation might be useful for realization of (2).

In Chpater 3, the gene of *P. taetrolens* Y-30 GS was over-expressed in *Escherichia coli,* which made it possible to supply large amounts of GS. Analysis of the gene revealed the occurrence of a modification system of GS in *P. taetrolens* Y-30. The modified GS was ineffective in theanine production, however, the recombinant GS formed in *E. coli* was hardly modified.

In Chapter 4, a new enzyme with much higher theanine-forming activity than *P. taetrolens* Y-30 GS was searched, and γ-glutamylmethylamide (GMA) synthetase (GMAS: Glu + methylamine + ATP → GMA + ADP + Pi) was found in an obligate methylotroph, *Methylovorus mays* No. 9. The enzyme showed high reactivity to ethylamine as well as methylamine, and formed about 400 mM theanine by the coupled fermentation with the 100% yield based on the substrates, Glu and glucose. An over-expression system of the GMAS gene was constructed.

In Chapter 5, the yeast fermentation of sugar under the condition of theanine production was investigated, which indicated that high concentration of EA together with an appropriate concentration of potassium phosphate buffer was effective to inhibition of ATP-consuming reaction(s) in yeast cells.

In summary, the present studies provided fundamental information for a new method of theanine production, and discussed practical utilization of the method.