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Theanine synthesis using γ- glutamyl transpeptidase from *Bacillus licheniformis* ER-15 and its estimation by a novel RP-HPLC method without derivatization

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Γ-glutamyl transpeptidase (GGT) is a well known enzyme for the synthesis of various γ-glutmayl compounds, and theanine is one of the most important neutraceutical among them. GGT gene from *Bacillus licheniformis* ER-15 was cloned and expressed in *E. coli* BL21 harboring pET 51b vector. Protein was purified by Ni^{2+}-NTA resin and biochemically characterized with pH and temperature optima to be pH 9 and 60°C respectively. Enzyme showed three times transpeptidase activity with respect to hydrolysis in case of glycylglycine as an acceptor while 1.5 times with ethylamine at 37°C. For the enzymatic synthesis of theanine, acetone precipitated enzyme was used which can be stored at 4°C with a shelf-life of more than 3 months. Various parameters for theanine synthesis viz. pH, donor and acceptor concentrations, enzyme concentration, time and temperature were optimized to be pH 9, 20mM glutamine, 200mM ethylamine, 0.75U/mL, 4h and 37°C respectively, in a 10ml reaction volume. Theanine was estimated using a novel RP-HPLC method without employing derivatization of theanine at 203nm. Above 80% conversion was obtained using above parameters. The process was then scaled up to 1L reaction volume and theanine was batch purified using Dowex 50W X 8 hydrogen form resin and eluted using ammonia water with a recovery of around 85%. Further optimization of theanine production was done by employing fed-batch method as the enzyme is stable at 37°C for more than 24h.

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