

## Cloning of glutamate decarboxylase and production of $\gamma$ -aminobutyric acid from *Lactobacillus brevis*

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**Background and Aims:**  $\gamma$ -aminobutyric acid (GABA) is a non-proteineaceous amino acid known to be a major inhibitory neurotransmitter in the mammalian brain tissues. GABA has several physiological functions such as neurotransmission, hypotensive activity and diuretic effects. In human GABA is synthesized by two isoforms of the pyridoxal 5'-phosphate-dependent enzyme glutamic acid decarboxylase (GAD65 and GAD67 in brain).

**Methods:** Some bacteria such as *Lactobacillus* strains (B1 and B2) were isolated from yogurt could produce GABA. Phylogenetic analysis based on the 16S rDNA sequence and biochemical studies were shown that they belong to the genus *Lactobacillus Brevis*. These strains were incubated in MRS medium containing 5% monosodium glutamic acid (MSG) at 30 °C for 48 h then produced GABA was analysed by HPLC. DNA fragments containing the GAD gene were obtained from NCBI. In order to clone the GAD gene from these strains, the PCR method was used for cloning. PCR was carried out using primers designed with Oligo6 software. The PCR product was purified from gel, ligated into T-vector by T4 DNA ligase, and then transformed into *E. coli* XL1Blue, then digested with restriction enzymes, the fragment was ligated into expression vector and was transformed into *E. coli* XL1Blue. The GAD gene was expressed in *E. coli* cells and the expression was confirmed by SDS-PAGE analysis and enzyme activity was assayed with Bromocresol green.

**Results:** The results from this study suggested that these strains could be used for the industrial production of GABA.

**Conclusions:** If oral GABA reaches the brain in any significant amount it could act as a tranquilizer. GABA as a neurotransmitter blocks nerve impulses and slows neuronal transmission and could affect anxiety and depression conditions. At least such strains will accelerate the development of functional fermented foods.

**Keywords:** GABA; Glutamate Decarboxylase; *Lactobacillus*; HPLC