ABSTRACT

The present study deals with the microbiological transformation of glutamate to γ-aminobutyric acid (GABA) from locally isolated bacterial species inhabited in cheese waste. Cheese waste samples were collected from different habitats i.e., local dairy shops, local pizza shops of Lahore, and from industrial area of Sahiwal, Pakistan, to isolate bacteria. Out of 40 isolates, 14 were evaluated for better GABA production ability. After primary and secondary screening, ISL-7 (32.7 mM) and ISL-15 (29.4 mM) were selected exhibiting better potential for GABA formation via submerged fermentation. ISL-7 was identified to be *Lactobacillus casei* by 16S rDNA gene fragment amplification and ISL-15 was also confirmed to be *Lactobacillus casei* by scanning electron microscopy. Various cultural parameters were optimized to enhance the production of GABA. A significant enhancement in GABA production was observed when the culture parameters including glutamate concentration as substrate (0.5%), initial pH (6.5), incubation time (48 h) and size of inoculum (8%), optimized. The thermophilic behavior and effect of stimulators and activators (casamino acid, thiamine HCl, n-butanol and glycerol) were evaluated on the GAD activity. Maximum activity was observed at a temperature of 35°C and casamino acid was found to be the stimulator of GAD activity (113.1 U/ml for ISL-7 and 100.4 U/ml for ISL-15). The role of micro minerals ($MgSO_4.7H_2O$, $MnCl_2$, $K_2Cr_2O_7$ and KI) and macro minerals (NH_4NO_3, $NaNO_3$, $CaCl_2$ and KH_2PO_4) on GABA production was also determined. The addition of 0.2 ml of $MgSO_4.7H_2O$ (4 mM), KI (0.5 mM), $CaCl_2$ (0.4%) and KH_2PO_4 (0.3% for ISL-7 and 0.2% for ISL-15) significantly enhanced the final production to 244 mM by ISL-7 and 232 mM by ISL-15. An overall enhancement of 7.46 fold for ISL-7 and 7.89 for ISL-15 was observed after optimizations of cultural conditions and additions of various stimulators, micro minerals and macro minerals, which is highly significant ($p \leq 0.05$).