

Testing the Effects of Vanillin on GABA Production in *Lactococcus lactis*

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Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter essential for stress regulation, mood stability, and anxiety reduction. Dietary factors such as artificial and flavors have been shown to influence GABA production by gut microbiota, highlighting a potential link between nutrition, microbial metabolism, and mental health. This study examines how vanillin, a previously untested and widely used food additive with antimicrobial properties, affects GABA production in *Lactococcus lactis* (*L. lactis*), a known GABA-producing bacterium and human probiotic. *L. lactis* was cultured in media containing glutamate and pyridoxal phosphate (PLP), precursors for GABA synthesis. OD595 measurements showed that vanillin inhibited bacterial growth in a dose-dependent manner, with a statistically-significant reduction between 0.1%, 0.3%, and 0.6% vanillin. To assess GABA production of the vanillin-treated *L. lactis*, *Caenorhabditis elegans* (*C. elegans*), a model organism, was used. Thrashing assays were performed to assess whether vanillin-treated *L. lactis* produced varying GABA concentrations as observed through *C. elegans* locomotion. Thrashing frequency decreased with increasing vanillin concentrations, suggesting elevated GABA levels. A statistically-significant reduction in thrashing was observed at 0.3% vanillin ($p = 0.0174$). These findings suggest that vanillin enhances GABA synthesis despite inhibiting bacterial growth, potentially contributing to reduced anxiety symptoms. This suggests a compensatory mechanism in *L. lactis* where individual bacterial cells may upregulate GABA production in response to vanillin exposure. Future studies will investigate how vanillin influences GABA production in mutant *L. lactis* to determine its effects on specific GABA pathways.

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