

In silico analysis of *gad* gene presence in classic and next-generation probiotics

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Introduction

Gamma-aminobutyric acid (GABA) is primarily produced in microbes through a **GAD enzyme-mediated decarboxylation** of L-glutamate, with pyridoxal 5'-phosphate (PLP) as a co-factor (Yogeswara et al., 2020). While studies have focused on pathogenic bacteria, **metagenomic analysis** shows **gut-colonising bacteria** also encode the *gad* gene.

The **GAD enzyme**, encoded by *gadA*, *gadB*, or **both**, mediates GABA synthesis in bacterial cells. Glutamate enters the cell via an antiporter channel for decarboxylation, and the final product, GABA, is secreted through a **glutamate/GABA antiporter**, encoded by the *gadC* gene (Iorizzo et al., 2023).

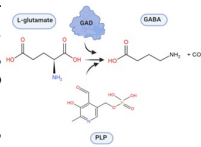


Figure 1 - Decarboxylation reaction of L-glutamate to GABA, catalysed by the microbial enzyme glutamate carboxylase

Objectives

To assess the capacity of four distinct species to produce and secrete GABA to the extracellular medium. The following species were selected for evaluation:

- *Levilactobacillus brevis*;
- *Lactocaseibacillus rhamnosus*;
- *Lactiplantibacillus plantarum*;
- *Akkermansia muciniphila*.

Methods

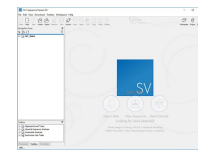
Sequence Search

Use of NCBI Database and Blast Tools



Sequence Alignment

Use of CLC Sequence Viewer



Results

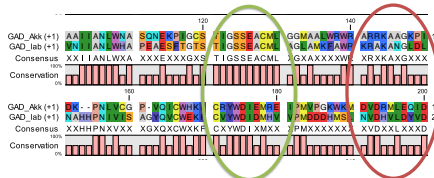


Figure 1 – Comparative analysis of sequences' polarity between LAB species and *A. muciniphila*

1. gene *gadC*:

Not found in *L. plantarum* and *L. rhamnosus* → Lack of specific transporter doesn't preclude secretion; GABA might be released via multifunctional transporters. *A. muciniphila* also lacked both sequences → Yet, it doesn't exclude GABA secretion.

2. *gadA* or *gadB*:

The *L. plantarum* strains had either *gadA* or *gadB*. Few strains of *L. rhamnosus* strains contained both sequences, lower GABA production ability. No matches for *gadA* or *gadB* were found in *A. muciniphila*, but it carries a decarboxylase and can potentially produce GABA.

3. Sequence alignment:

Both sequences showed dissimilarity, but performed similar functions due to key active centres. The existence of similar domains supports these assumptions.

Conclusions

All selected species exhibited the **capacity to produce GABA** to some extent. *Levilactobacillus brevis* is the only species to contain the **entire GAD system**, while *L. rhamnosus* and *L. plantarum* contain only **one of the encoding genes**.

Akkermansia muciniphila, a Next-Generation Probiotic, presents a **decarboxylase encoding gene**. Nevertheless, the decarboxylase enzyme displays **considerable variation** between different LAB species and *A. muciniphila*.

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References

Iorizzo, M., Paventi, G., Di Martino, C., 2023. Biosynthesis of Gamma-Aminobutyric Acid (GABA) by *Lactiplantibacillus plantarum* in Fermented Food Production. *Curr Issues Mol Biol* 46, 200–220. <https://doi.org/10.3390/cimb46010015>

Yogeswara, I.B.A., Maneerat, S., Haltrich, D., 2020. Glutamate Decarboxylase from Lactic Acid Bacteria—A Key Enzyme in GABA Synthesis. *Microorganisms* 8, 1923. <https://doi.org/10.3390/microorganisms8121923>