Assessing the GABA production ability of bacteria of food interest using rapid and simple colorimetric method

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Context & objective

In recent years, consumer's demand for healthy food products have increased considerably. As a result, much research is dedicated to the development of functional foods, i.e. food containing valuable bioactive compounds. Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the human brain and is involved in many physiological mechanisms such as anxiety management or blood pressure lowering. Supplementary GABA intake through food could thus be of interest for consumer's health. Many microorganisms of food interest have been reported to produce GABA indicating that its content can be increased by microbial fermentation. Indeed, GABA production from L-glutamate is a bacterial mechanism to maintain pH homeostasis. The objective of the present work was thus to identify GABA-producing Lactic acid Bacteria and Propionic Bacteria of food interest, using a simple colorimetric method exploiting this mechanism.

Strategy

Colorimetric screening of GAD activity under acid stress

- GABA production in bacteria relies on glutamate decarboxylase (GAD) activity
- Bacteria are under **resting cell conditions** in a reaction medium containing **L-glutamate as sole substrate** and **Bromocresol green** (BCG), a pH indicator.

① In acid condition, BCG is under green (protonated) form

② In bacteria with GAD activity : GABA is produced from L-glutamate → H+ consumption

③ pH increases → BCG changes to blue (deprotonated) form

→ **Supernatant color change** : indirect marker of **GABA production ability**

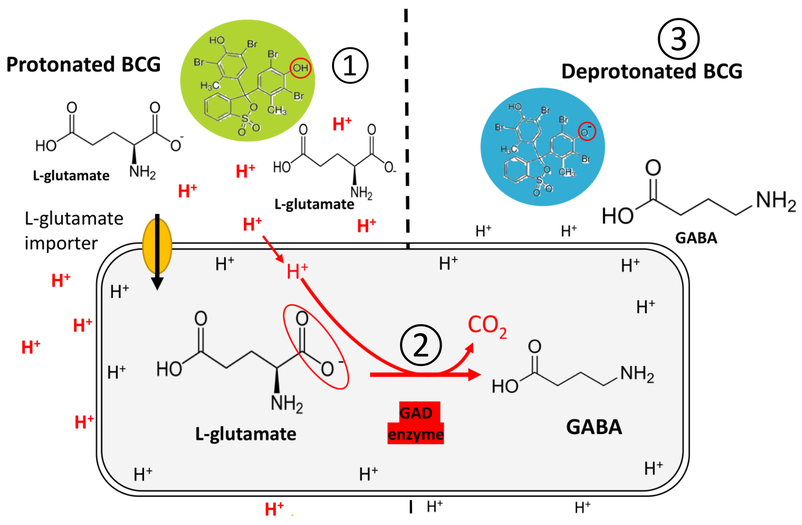


Figure 1. Principle of the colorimetric method used in the present work and first described by ***Cotter* et al. *2001a***.

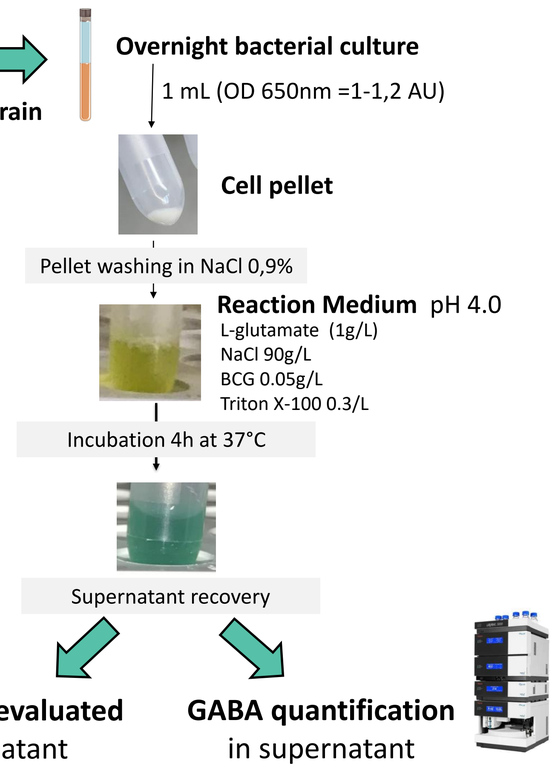
Experimental design

106 bacterial strains

8 species of food interest (GRAS status)

Species	n =
Lactococcus lactis (LC)	14
Lactobacillus helveticus (LH)	6
Lactobacillus reuteri (LR)	3
Levilactobacillus brevis (LB)	9
Lacticaseibacillus paracasei (LPC)	13
Lactiplantibacillus plantarum (LP)	21
Propionibacterium freudenreichii (PF)	20
Streptococcus thermophilus (ST)	20

n= 3 per strain



2 parameters evaluated
- pH in supernatant
- Color change

GABA quantification in supernatant (HPLC)

Results

How the screening method discriminate the tested strains ?

GAD activity of the strains based on colorimetric screening

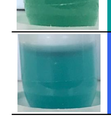
Color observed	Interpretation	Mean pH	[min-max]
	*Control condition –no GAD activity*	*4.08*	*[4.02-4.1]*
	No or weak GAD activity	4.13	[4.03-4.44]
	Intermediate GAD activity	4.75	[4.52-5.07]
	Strong GAD activity	5.32	[4.93-5.79]

Table 1. Color change observed after 4h incubation and interpretation regarding GAD activity. Mean pH value (as well as minimal and maximal values) are given based on all the strains assigned to the same color group.

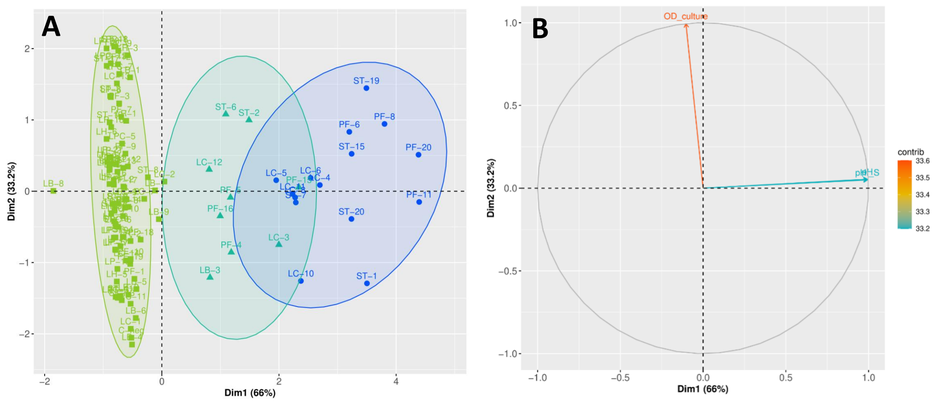
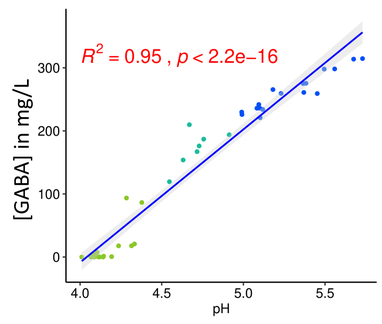


Figure 2. Principal component analysis of screening data. Mean of 3 replicates per strain were used for the analysis. (**A**) Individual plot showing strains repartition on dimensions 1 and 2 - Color group correspond to the color change observed in the supernatant after incubation.(**B**) Variable plot showing the contribution of the evaluated parameters on the same dimensions

- Strains were assigned into 3 groups based on supernatant color change during incubation suggesting 3 increasing levels of GAD activity.
- The strain distribution is mainly correlated with the supernatant pH (Dimension 1).
- Strains similar to control strain (green group) are clearly separated from other bacteral strains, while the two other groups (Blue-green and Blue) corresponding to higher GAD activity levels slightly overlap.

Are screening results correlated with [GABA] ?



- A good correlation was observed between color, pH and GABA production in supernatants

Figure 3 Pearson correlation plot between supernatant pH values and and GABA concentration obtained by HPLC. Data from 40 strains representatives of the different color groups were used for the analysis.

GABA quantification in microbial supernatants

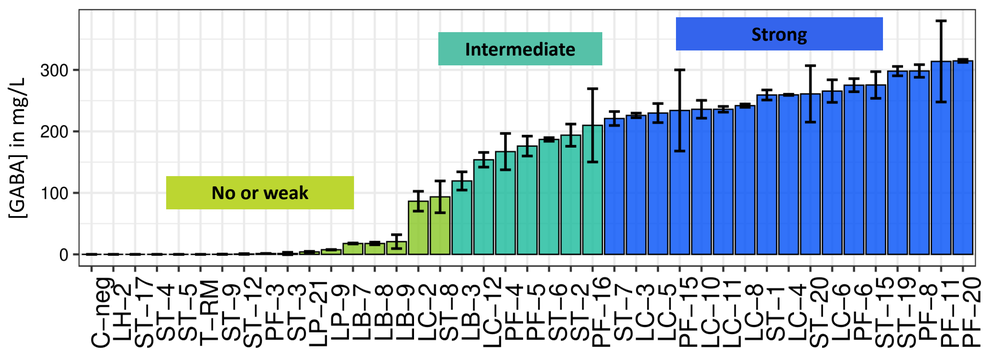


Figure 4. GABA concentration in bacterial supernatants quantified by HPLC. Forty strains, belonging to the three « color groups », previously identified were analysed in triplicates. « C-neg » and « T-RM » correspond to a non GABA-producing strain used as control and the reaction medium control respectively.

- Strains that do not produce GABA and those that did but at low concentration were not discriminated based on color change
- GABA production level between the three groups was observed: none or weak (<100 mg/L), intermediate (between 100 and 200 mg/L) and strong GABA production (>200 and up to 300 mg/L)

GABA production among bacterial species

Screening results consolidated by GABA quantification

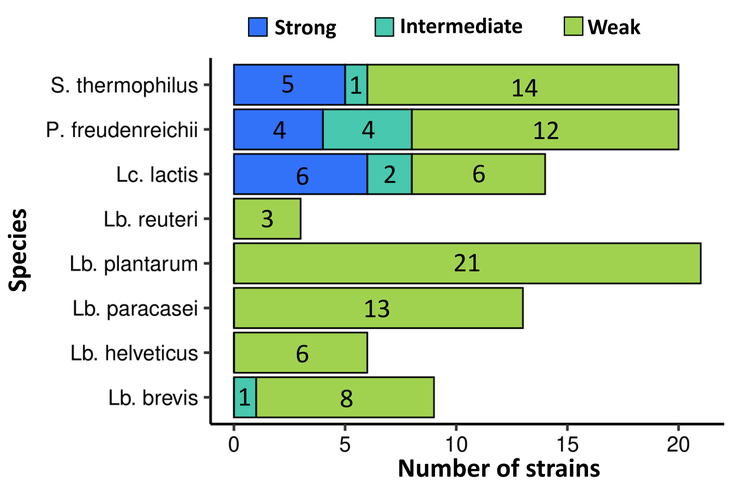


Figure 5. Repartition of the GABA-producing ability within each species.

- GABA production ability is species-dependent: 4 species out of 8 were shown to produce GABA
- GABA production within species was also strain-dependent. Not all strains were able to produce GABA or to reach the same level of production
- Overall, 21.7% of the tested strains were identified as GABA-producers

Conclusion

This colorimetric screening allowed to assess rapidly the GABA production ability of more than 100 bacterial strains. The method was shown to differenciate between three levels of GABA production within species based on GAD enzyme activity and was confirmed by GABA quantification.

Altogether this work allowed to efficiently identify 15 bacterial strains (from four species) with the strongest GABA-production ability and constituted a first step towards the development of GABA-enriched fermented dairy products.

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https://stlo.rennes.hub.inrae.fr/

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