

Evaluation for optimal conditions of γ -aminobutyric acid production by *Lactiplantibacillus plantarum* ST0414

Kayque Ordonho Carneiro¹, David Fernando dos Santos², Eduardo Purgatto², Svetoslav Dimitrov Todorov^{1,3}

¹. USP, Universidade de São Paulo, Av. Prof. Lineu Prestes, 580 - Butantã, São Paulo - SP, 05508-000;

². FoRC, Food Research Center, Lab. de Eng. de Alimentos, Ed. Semi Industrial - R. do Lago, 250 - bloco C - Cid;

³. CISAS, Instituto Politécnico de Viana do Castelo, 4900-347 Viana do Castelo, Portugal;

Lactic acid bacteria are known for their beneficial properties, including production of various antimicrobials and health promoting metabolites, such as γ -aminobutyric acid (GABA), a non-protein amino acid that acts as a neurotransmitter, produced mainly through the decarboxylation of glutamate by the enzyme glutamate decarboxylase. *Lactiplantibacillus plantarum* is a species applied in the fermentation of dairy, meat and plant-based products, where plays an essential role as starter, adjunct, protective or probiotic/postbiotic culture, increasing functional properties in the synthesis of its metabolites, such as GABA.

The objective of this study was to evaluate safety and GABA production by *Lpb. plantarum* ST0414 and explore influence of the culturing conditions such as cell density of the producer, concentration of monosodium glutamate (an GABA precursor), pH, temperature and incubation time. Expressed GABA was quantified by gas chromatography coupled to mass spectrometry (GC-MS).

In this study we have studied *Lpb. plantarum* ST0414 strains, isolated from Bulgarian white brine cheese produced by medium scale producers and identified based on its biochemical and genetic characteristics including 16S rRNA sequencing. The strain ST0414 was selected for current study based on preliminary screening for GABA production between more than 150 bacterial isolates, most of them obtained from dairy products. GABA production from *Lpb. plantarum* ST0414 was evaluated in different levels of cellular density (105 -108

CFU/ml), monosodium glutamate concentration (30 mM-120 mM), pH (4.0-8.0), temperatures (from 30°C to 50°C) and incubation time (24h-120h), varying one method at a time, maintaining other standard conditions of 106 UFC/ml, 60 mM, pH 6.0, 37°C and 24h and following to additional experimental set-ups. Cell free supernatant was obtained after centrifuged at 15.000×g for 15 minutes at 20°C, diluted 10×, derivatized in triplicate with methyl chloroformate and quantified by GC-MS.

Comparing results obtained from the different experiments following preselected variables, the production of GABA by *Lpb. plantarum* ST0414 was present in superior levels when was subjected to a longer incubation time 120h (27.98 mg/ml) and microbial density of 105 CFU/ml (16.13 mg/ml), respectively. Extreme temperatures of 30°C and 50°C used demonstrated better results (13.64 mg/ml and 14.27 mg/ml, respectively), being assimilated to the greater production of GABA under stress conditions by the ST0414 strain. Variations in pH and glutamate concentration did not prove to be influencing factors in the production of GABA, maintaining close values between their respective variations. Moreover, based on

performed biochemical and physiological tests, hemolytic, gelatinase, proteolytic and lipolytic activity, biogenic amines production and antibiotic resistance, including different van genes, *Lpb. plantarum* ST0414 can be considered as a safe strain.

Obtained results, suggest that GABA production can be increased when *Lpb. plantarum* ST0414 is exposed to stress growth conditions. In current study we have recorded that *Lpb. plantarum* ST0414 has optimal conditions for GABA production with 120h of incubation, cell density of 105 UFC/ml and temperature of 30°C or 50°C. Thus, information can be further applied and scaled with aim of biotechnological production where *Lpb. plantarum* ST0414 can be explored as industrial GABA producer.

References

- RAWLINSON, C. et al. A rapid method for profiling of volatile and semi-volatile phytohormones using methyl chloroformate derivatization and GC–MS. *Metabolomics*, v. 11, n. 6, p. 1922–1933, 1 dez. 2015.
- ZHANG, Q. et al. Characterization of γ -aminobutyric acid (GABA)-producing *Saccharomyces cerevisiae* and coculture with *Lactobacillus plantarum* for mulberry beverage brewing. *Journal of Bioscience and Bioengineering*, v. 129, n. 4, p. 447–453,
- ZHUANG, K. et al. Transcriptomic response to GABA-producing *Lactobacillus plantarum* CGMCC 1.2437T induced by L-MSG. *PLOS ONE*, v. 13, n. 6, 1 jun. 2018.

Acknowledgements: This research was partially by the Centre for Research and Development in Agrifood Systems and Sustainability, funded by FCT (UIDB/05937/2020 and UIDP/05937/2020), Fundação para a Ciência e a Tecnologia, Portugal; by FAPESP, processo 2023/14944-2, 2023/05394-9, São Paulo, SP, Brazil