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PROTEOMICS DISCLOSE THE EFFECT OF CARBON AND NITROGEN SOURCES ON GABA PRODUCTION BY *Levilactobacillus brevis* CRL 2013

Cataldo PG, Rios Colombo NS, Savoy de Giori G, Saavedra L, Hebert EM
Centro de Referencia para Lactobacilos (CERELA- CONICET)- Tucumán, Argentina. Email: pcataldo@cerela.org.ar

Gamma-aminobutyric acid (GABA) is a non-protein amino acid, which functions as the main inhibitory neurotransmitter in humans showing potential for improving several mental health conditions such as stress and anxiety. The microbiota-gut-brain axis is a bidirectional communication pathway between the central nervous system and the gut microbiota, which is mediated by several direct and indirect stimuli. Microbial GABA synthesis within the gut can affect host mental health outcomes. In bacteria, GABA is produced and released by the glutamate decarboxylase (GAD) system, which consists of three key elements: the positive transcriptional regulator (GadR), the glutamate/GABA antiporter (GadC) and the glutamate decarboxylase enzymes (GadA and/or GadB). Understanding the molecular characteristics of GABA production by the microbiota can provide insights into new therapies for mental health. Therefore, the aim of this study was to assess the effect of different nitrogen; yeast extract (YE) and casitone (C); and carbon (hexose and pentose) sources on the fermentation profile and GABA production by the efficient GABA producer, *Levilactobacillus (L.) brevis* CRL 2013 strain and explore the associated proteomic changes. GABA accumulated up to 72 h in glucose and fructose- CDM (CDMGF) supplemented with YE and C; this was related to a reduction in glutamate concentration and an increase in the extracellular pH. Lactic acid, acetic acid, and ethanol (2.5 g/L) could be detected in the fermented medium. In CDM-Xylose (CDMX), the cell density was markedly higher than in CDMGF, presenting the highest values of lactic (5.6 g/L) and acetic (3 g/L) acids while ethanol was not detected. Moreover, GABA production decreased about 13 times and the amount of residual glutamate was significantly higher (9 times) with respect to the CDMGF. The initial addition of ethanol to the CDMX increased both GABA production and the levels of organic acids. The proteomic data revealed that GadA was upregulated in CDMGF in the presence of YE and C (294 and 50 times, respectively). Under these conditions, GadB expression remained unchanged, whereas CcpA and HPr kinase were upregulated after YE and C supplementation (3.7 and 2-fold respectively). Furthermore, YE and C supplementation in the CDMGF induced the differential expression of proteases and peptidases. These expression trends were confirmed by transcriptional assays (RT-qPCR) with *recA* as the housekeeping gene. Additionally, ethanol supplementation increased *gadA* expression in the CDMX. Our results expand knowledge about the regulation of the GAD system in lactic acid bacteria, where carbon and nitrogen sources as well as some fermentation by-products may play a key role and support the use of *L. brevis* CRL2013 as a microbial cell factory for the efficient production of GABA using alternative energy sources.

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ANALYSIS OF THE EXPRESSION AND REGULATION OF A TYPE I-F CRISPR-CAS SYSTEM

Molina MC, Quiroga C.
Universidad de Buenos Aires, Facultad de Medicina, IMPaM-CONICET, Argentina.
E-mail: molinamariacarolina@gmail.com

CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats and its associated proteins) systems are considered the prokaryotic adaptive immune system responsible for defending the host against mobile elements. They exist in nature with remarkable diversity, depending on a single protein or complexes of multi-effector Cas proteins. Among the multi-subunit complexes, the Type I-F is able to seek and destroy DNA through a surveillance complex (Csy) and a nuclease (Cas2/3). The overall goal of this work is to study the conditions that play a role in the regulation of the Type I-F CRISPR-Cas system of *Shewanella xiamenensis* Sh95 which is composed of 6 genes *cas1-cas2/3-csy1(cas8f)-csy2(cas5f1)-csy3(cas7f1)-csy4(cas6f)* followed by a CRISPR array of 152 spacers. We observed that *cas* genes are transcribed as a polycistronic operon during stationary phase. In addition, we performed a predictive *in silico* analysis of the upstream region of *cas1* and the entire *cas* operon using BPROM, CNNProm, BacPP, and Virtual Footprint tools. Several putative promoter sequences and transcription factors binding sites were predicted for both regions. Binding sites for LexA, H-NS, ArgR, and RpoD were detected upstream of *cas1*. Moreover, an IS*256* was identified upstream of the *cas* operon by ISfinder and BLAST. Promoter prediction revealed the presence of H-NS and LexA binding sites within this IS, which might have added complexity to the regulation of this system. We also tested these regions for a possible posttranscriptional regulation against the Rfam database and we did not find any predicted family of ncRNAs involved. Next, we tested and verified the effect of different stress treatments for *S. xiamenensis* Sh95. We analyzed osmotic stress (20% sucrose, 40 min) and nutrient deprivation stress (culture in M9 minimal medium for 2 h) by monitoring the bacterial growth (OD_{600nm}) and viability (CFUs/mL) for validation of these experiments. In osmotic stress, we observed a decrease in OD_{600nm} relative to T_0 with an increase in the concentration of viable cells proportionally to untreated samples, indicating a decrease in cell size by plasmolysis without affecting cell division. In nutrient deprivation treatment, we observed small changes in OD_{600nm} and a constant rate count of CFUs/mL which would be associated with a temporary arrest in cell division. Exposure to UV light stress (254 nm, 30 J/m², sampled periodically) was evaluated by the viable counts and the DNA damage effect for up to 300 seconds monitoring the activation of the SOS response and the levels of *lexA* and *recA*. We quantified the effect of these stress experiments on the transcription levels of *cas1* and *csy4* by RT-qPCR. Finally, our results will provide insights into induction and repression conditions of Type I-F CRISPR-Cas systems contributing to a better understanding of its regulation scenario, which still remains unclear.