Mahidol University Faculty of Environment and Resource Studies
ENVI MAHIDOL ENRIC
The 6th Environment and Natural Resources International Conference (ENRIC 2026)
Theme: Real World Impact: Resilience for a Changing Climate
24 - 25 November 2026, Bangkok, Thailand

Upcycling of a Soy Sauce Industry Byproduct into High-value Glutamic Acid Derivatives via Engineered *Halomonas elongata* Cell Factories

Pulla Kaothien-Nakayama[1], Hanna Mitsunaga[1], Ziyan Zou[2], Huynh Cong Khanh[2,3], Hiroyuki Tamino[4], and Hideki Nakayama[1,2,*]

[1]Graduate School of Integrated Science and Technology, Nagasaki University, Nagasaki City, Nagasaki 852-8521, Japan
[2]Graduate School of Fishery and Environmental Sciences, Nagasaki University, Nagasaki City, Nagasaki 852-8521, Japan
[3]College of Environmental and Natural Resources, Can Tho University, Can Tho, Vietnam
[4]Maruisyouyu Company, Limited, 1-1-5 Nishi, Nakano City, Nagano 383-0021, Japan
[*]Corresponding Author E-mail: nakayamah@nagasaki-u.ac.jp

ABSTRACT

Soy sauce (*shoyu*), a staple of Japanese cuisine, is produced by fermenting a mixture of soybeans, wheat, salt, and water with koji molds. Each year, Japan's soy sauce industry generates approximately 110,000 tons of a byproduct known as soy sauce cake (*shoyu kasu*), which is recycled and reused only to a limited extent due to its high salinity. *Halomonas elongata* GOP, isolated in our laboratory, is a mutant halophilic bacterium capable of assimilating nutrients from a broad range of biomass sources and thriving in high-salinity environments containing 1 M NaCl by accumulating glutamic acid as its major osmolyte. Using this strain as a host, we generated two metabolically engineered strains, *H. elongata* ZN3 and HN10, which overproduce and accumulate the high-value glutamic acid derivatives γ-aminobutyric acid (GABA) and L-proline (Pro), respectively. Owing to their genetic backgrounds, *H. elongata* ZN3 and HN10 can also assimilate nutrients from a diverse range of biomass sources and thrive in high-salinity environments containing 1.2 and 1.37 M NaCl, respectively.

To develop a novel method for recycling soy sauce cake, we evaluated the ability of *H. elongata* ZN3 and HN10 to utilize soy sauce cake lysate as the sole nutrient source to support cellular growth and facilitate the biosynthesis of GABA and Pro. Soy sauce cake, supplied by Maruisyouyu Company (Nagano, Japan), was subjected to alkaline hydrolysis in 1.25 M NaOH at 121°C for one hour. Following neutralization with HCl, the lysate was filtered, and its volume was adjusted to obtain a final NaCl concentration of 1 M. This preparation, designated soy sauce hydrolysate (SH) medium, was subsequently used to cultivate *H. elongata* ZN3 and HN10. The culture data demonstrated that SH medium adequately supported the growth of both strains and, as determined by HPLC, enabled the production of GABA and Pro at levels comparable to those observed in cells cultivated in synthetic M63 medium. Notably, both the growth rate and cell yield of *H. elongata* ZN3 were significantly higher when the strain was cultured in SH medium than when it was cultured in M63 medium. This result may be attributable to the unique nutrient composition of the SH medium, which is rich in the precursor glutamic acid but contains only a small amount of glycine betaine, a compound known to interfere with the induction of the salt-inducible *ectA* promoter that controls the expression of the GABA and the Pro biosynthesis operons in *H. elongata* ZN3 and HN10.

Keywords: *Halomonas elongata*/ Soy sauce cake/ High salinity/ Industrial waste/ Bioproduction/ GABA/ Proline