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Enhanced Production of Poly-γ-glutamic Acid (γ-PGA) by *Bacillus velezensis* Mutant Induced via Pin-to-plate Atmospheric Cold Plasma

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Abstract

Poly-gamma-glutamic acid (γ-PGA) is a biodegradable, microbially derived polymer with wide-ranging applications in the food, pharmaceutical, agricultural, cosmetic, and wastewater treatment industry [1]. Industrial-scale production, however, requires high-yielding microbial strain to develop an economically viable process [2]. In this study, pin-to-plate atmospheric cold plasma (ACP) was investigated as a novel, non-thermal physical mutagenesis tool to enhance γ-PGA production in *Bacillus velezensis*. The wild-type strain was exposed to ACP treatment (170 V-230 V; 2-8 min), and a genetically stable mutant (*Bacillus velezensis* 230*V6M*) was isolated for comparative shake-flask fermentation. One-factor-at-a-time (OFAT) experiments were performed to optimize the fermentation parameters (incubation time, temperature, pH, and inoculum size). Mathematical modeling of fermentation kinetics using Logistic, Weibull, and Luedeking-Piret equations demonstrated that ACP-induced mutagenesis significantly increased the rate of biomass formation, γ-PGA synthesis, and substrate consumption (glucose, citric acid, and monosodium glutamate) in the mutant relative to the wild type. The mutant achieved a maximum γ-PGA titer of 64.84 ± 0.66 g/L at 84 h, resulting in a 49.30 % increase over the wild-type strain (43.44 ± 0.94 g/L at 96 h), with a maximum viable cell count of 10.28 Log CFU/mL. Consistently, the mutant strain exhibited a maximum broth viscosity of 325.36 ± 1.70 mPa.s in contrast to 174.58 ± 1.91 mPa.s for the wild-type strain, indicating enhanced γ-PGA accumulation. The weight-average molecular weight of γ-PGA produced by the mutant was 588.83 ± 29.10 kDa compared with 478.77 ± 20.30 kDa for the wild-type strain after 84 h of fermentation. The LC-MS/MS-based metabolic profiling of spent media confirmed distinct alterations in the extracellular metabolite secretions of both the strains; notably, the mutant secreted higher titers of metabolites involved in γ-PGA biosynthetic pathway. Structural characterization of γ-PGA using FT-IR and ^{1}H NMR confirmed that ACP mutagenesis did not modify the primary chemical structure of the biopolymer. Collectively these results demonstrate that ACP mutagenesis is an effective, environmentally safe mutagenic tool for enhancing microbial metabolite production, offering a safer alternative to conventional physical and chemical mutagens.

References

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