

OPTIMIZING BIOMASS AND SUPEROXIDE DISMUTASE IN *Tetraselmis chuii* THROUGH STRESS INDUCTION AND ADAPTIVE LABORATORY EVOLUTION

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Microalgae are promising natural antioxidant resources due to their adaptation to oxidative stresses in harsh oceanic conditions. Limited studies have explored the correlation between antioxidant activities and nutrients or exogenous factors that elevate superoxide dismutase (SOD) levels. *Tetraselmis chuii*, a marine microalga with high SOD bioactivity, was recently listed as a novel food by the EU Commission. This study aimed to, at first, optimize the production medium to enhance biomass and SOD production in *T. chuii*, followed by stress induction to further boost SOD bioproduction. The Adaptive Laboratory Evolution (ALE) technique was employed to adapt *T. chuii* under stressed conditions for SOD enrichment. A two-stage cultivation process was designed: the first stage involved statistical optimization of the cultivation medium to increase biomass, while the second stage applied various stresses at the late exponential growth phase to induce oxidative stress and trigger SOD production as a defense mechanism. Among several media, the F medium was selected as the basal medium, and NH₄Cl was identified as the optimal nitrogen source. Significant medium components (NH₄Cl, NaH₂PO₄, CuSO₄, and MnCl₂) were screened using a 2^{k-n} factorial design and optimized via Response Surface Methodology (RSM). The optimized medium, containing 0.21 g/L NH₄Cl, 0.15 g/L NaH₂PO₄, 0.412 mg/L CuSO₄, and 0.04 mg/L MnCl₂, increased *T. chuii* biomass from 416.67 mg/L to 564.44 mg/L and SOD activity from 1479.23 U/g to 2757.27 U/g biomass. Subsequently, different stresses, including heavy metal (CuSO₄), oxidant agents, salinity, and nitrogen starvation, were applied to enhance SOD production further. CuSO₄ at 5.0 mg/L induced the highest SOD activity (5774.76 U/g biomass), showing copper's potential in boosting SOD production. A 27-week ALE experiment successfully increased SOD activity in *T. chuii* to 1647.64 U/g biomass, compared to 1021.28 U/g in non-stressed strains.

Keywords: Adaptive laboratory evolution, environmental stressors, nutrient optimization, Superoxide dismutase, *Tetraselmis chuii*