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ISOLATION AND PHYSIOLOGICAL CHARACTERIZATION OF AN L-METHIONINE-OVERPRODUCING BACTERIAL STRAIN FROM INDUSTRIAL WASTEWATER

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1. Introduction

L-methionine is a high-value, sulfur-containing essential amino acid widely used as a feed additive in livestock production and as a key ingredient in pharmaceutical and cosmetic formulations. While chemical synthesis yields a racemic DL-mixture, fermentation-based production offers a cleaner, more sustainable alternative — one that selectively delivers only the biologically active L-enantiomer. Industrial environments — particularly the soils surrounding manufacturing facilities and their associated wastewater systems — represent some of the harshest ecological niches found in nature. Persistent chemical pollution, heavy organic loading, and fluctuating sulfur gradients create intense selective pressure on resident microbial communities. The bacteria that manage to thrive under these conditions often develop remarkable metabolic adaptations, including an enhanced capacity to overproduce amino acids. With this in mind, the present study undertakes a systematic effort to isolate, molecularly identify, and physiologically characterize wild-type bacterial strains recovered from industrial soils and manufacturing plant effluents — with the ultimate goal of establishing a scalable bioprocess for extracellular L-methionine production.

2. Materials and Methods

Bacterial isolates were selectively recovered from industrial soil core samples and liquid effluent collected at active drainage points of manufacturing facilities. To assess their technological and physiological potential, a purpose-built minimal cultivation medium — designated Medium No. 2 — was formulated. The medium was specifically designed to amplify sulfur metabolic flux, incorporating ammonium sulfate ($(NH_4)_2SO_4$, 5.0 g/L) as a combined nitrogen and sulfur source, alongside D-glucose (20.0 g/L) as the primary carbon and energy substrate. Fermentation was carried out over a 96-hour window at 30°C on an orbital shaker running at 180 RPM. Growth kinetics were tracked continuously via optical density measurements at 600 nm (OD_{600}) using a UV-Vis spectrophotometer, while glucose consumption and culture pH were monitored dynamically throughout. Extracellular amino acid accumulation was quantified by High-Performance Liquid Chromatography (HPLC) following removal of bacterial biomass through high-speed refrigerated centrifugation.

3. Results and Discussion

Screening of eight bacterial isolates from industrial soils and wastewater canals revealed considerably different growth and amino acid secretion of the isolates in ammonium sulfate-amended medium. Such a physiological turning point approximately occurs during the late exponential and

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stationary phases at 48-72 hours of cultivation. In this context, Isolate-1, the most promising overproducer of all the isolated strains, was isolated from an industrial wastewater discharge point. Showing a high biomass productivity and a strong secretion rate of amino acids, quantitative HPLC measurements indicated that a total of 0.783 mg/g pure L-methionine was secreted into the culture supernatant of Isolate-1, indicating active methionine secretion in this strain. The custom designed Medium No. 2 helped the predominant isolates to eliminate their lag phase, and promoting good viability for long times of culture, even in the face of the progressive sulfate accumulation in the culture medium that markedly decreases the viability of old cultures. The presence of stabilizing agents $CaCl_2$ (0.01 g/L) and $MgSO_4$ (0.5 g/L) protects the membrane from rupture during osmotic pressure. The presence of methionine in the supernatant and its absence in the cells indicates that Isolate-1 has an active membrane efflux mechanism, which could be useful for further research. These specialized amino acid exporter pumps seem to constantly excrete the newly synthesized methionine out of the cytoplasm and prevent the formation of an intracellular methionine pool that would enable feedback inhibition. This allows for unimpeded methionine overproduction because the transporting machines to extend the metabolic pathway are always being pushed.

4. Conclusion

This study successfully established a practical workflow for isolating and evaluating wild-type bacterial strains with high L-methionine production. The custom culture device used in this workflow is low-cost. The Isolate-1 strain, which was screened from an industrial waste habitat, can secrete 0.783 mg/g of L-methionine into the culture supernatant. This secretory production model eliminates the costly and labor-intensive cell lysis step, greatly simplifies downstream processing, and provides a solid, industrially feasible foundation for subsequent bioprocess development. Looking ahead, future work will focus on the molecular identification of Isolate-1 via 16S rRNA gene sequencing, followed by a stepwise scale-up in laboratory bioreactors to assess its performance under pilot-scale conditions.

Keywords: L-methionine, Industrial soils, Manufacturing wastewater, Ammonium sulfate minimal medium, Extracellular secretion, HPLC validation.

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