

## Abstract

Keratin is a structural protein found in feathers, wool, and hair and has diverse applications in cosmetics and pharmaceuticals. While conventionally extracted using harsh chemicals, these methods pose environmental risks due to toxic waste generation. Microbial fermentation, particularly using *Bacillus subtilis*, presents a sustainable alternative, but its quality and efficiency remain lower than chemical extraction. This study aims to optimize submerged fermentation (SmF) for green keratin extraction by evaluating five *Bacillus* sp. strains of varying inoculum percentages. The fermentation broth will be supplemented with chicken feathers as the primary substrate. Bacterial growth will be monitored through optical density (OD600) measurements, while feather degradation efficiency will be assessed by quantifying the remaining feather mass over time. Protein yield will be evaluated using the BCA assay, with SDS-PAGE and FTIR spectroscopy employed to analyze the quality of the extracted keratin. By improving SmF efficiency, this research seeks to enhance the viability of fermentation-based keratin extraction as an eco-friendly industrial alternative. The top strain (N10ND) achieved 27% feather weight reduction after 3 days of fermentation while producing 170.5 mg of protein (17% yield). SDS-PAGE analysis revealed distinct bands in the 11-20 kDa range, consistent with expected  $\beta$ -keratin fragments. FTIR spectra showed a notable shift of the Amide A band from  $3280\text{ cm}^{-1}$  to  $3200\text{ cm}^{-1}$  upon fermentation, indicating increased N-H hydrogen bonding. The Amide I peak remained near  $1633\text{ cm}^{-1}$  ( $\beta$ -sheet keratin). These results confirm partial solubilization of feather keratin with preservation of its core  $\beta$ -keratin structure.

**Keywords:** Green Extraction, Keratin, Submerged Fermentation