

# Chapter 1

## Introduction

### 1.1 Background

Keratin is a structural protein found in feathers, hair, wool, and nails, and it is widely used in industries such as cosmetics and pharmaceuticals. Traditionally, keratin has been used for hair treatments, animal feed, and fertilizers. However, recent advancements highlight its potential applications in the medical field, due to its biocompatibility and biodegradability (Rajabi et al., 2020). While hydrolyzed keratin suffices for cosmetic and agricultural use, therapeutic fields such as tissue engineering and regenerative medicine demand high-purity keratin with intact secondary structures (Soleymani & Karbasi, 2024). This growing demand has underscored the need for extraction techniques capable of preserving the native structure of keratin proteins, enabling their use in biomedical applications.

Typically, keratin is extracted from slaughterhouse waste using strong chemicals such as sodium hydroxide, sulfides, and oxidizing agents. While effective, these methods pose significant environmental hazards, including toxic waste generation and water pollution (Wang & Tong, 2022). To address these environmental concerns, microbial fermentation using keratinolytic microorganisms like *Bacillus subtilis* has emerged as a sustainable alternative. These microbes naturally degrade keratin through enzymatic action, reducing reliance on harsh chemicals. However, a significant drawback of this approach is that the yield and quality of keratin obtained through fermentation are currently lower than those of chemical extraction (Cassoni et al., 2018). Therefore, optimizing fermentation parameters is crucial to enhance its efficiency and make it a viable industrial alternative.

This study focuses on improving submerged fermentation (SmF) for keratin extraction by testing five *Bacillus subtilis* strains and varying inoculum percentages. The experiment will involve preparing a fermentation broth supplemented with chicken feather substrate. Submerged fermentation will be carried out using five different strains with varying inoculum percentages to assess their impact on keratin degradation. Feather hydrolysis efficiency will be evaluated by measuring the amount of

undegraded feathers in the substrate at different time intervals. Bacterial growth and metabolic activity will be monitored by measuring the OD600 and pH levels. The total protein concentration in the fermentation broth will be measured using a BCA reagent. SDS-PAGE and FTIR spectroscopy will characterize the quality of extracted keratin, ensuring its potential suitability for biomedical applications.

## 1.2 Objective

This study aims to optimize the yield and quality of keratin extracted from chicken feathers by screening five different *Bacillus* strains for their keratinolytic potential. The effect of varying inoculum percentages in submerged fermentation will also be evaluated to determine the optimal conditions for maximum keratin degradation.

## 1.3 Hypothesis

$H_0$  (Null hypothesis)

- Different strains does not have a significant difference in keratin extraction.
- Varying inoculum percentage does not have a significant effect on keratin extraction.

$H_1$  (Alternative hypothesis)

- Different strains have a significant difference in keratin extraction.
- Varying inoculum percentage does have a significant effect on keratin extraction.