USE OF AGRO-INDUSTRIAL WASTE - USE OF FARM FEATHERS FOR THE PRODUCTION OF PROTEINS AND ENZYMES

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ABSTRACT

Chicken meat per capita consumption reaches 45 kg annually, and the annual output is approximately 14 million tons. Feathers are a poultry product residue that has slow environmental degradation. Their recalcitrance is due to their composition with approximately 90% of keratin, a fibrous and insoluble protein that, when properly processed, can be converted into products with commercial value. Faced with the need for innovative and sustainable solutions for the use of industrial poultry feathers and the need to offer sustainable inputs for the textile industry, this research proposes the use of keratinase-producing microorganisms to degrade the structure of feathers and their reuse in the formation of fibers with the textile application and for the production of keratinases.

Keywords: Feather keratin. Chicken feather. Keratinase. Biodegradation.

1 INTRODUCTION

The development style of today's society is generating a rapid process of environmental erosion, affecting the biosphere and the availability of renewable and non-renewable natural resources around us and overloading the planet. One example of industrial activity that generates worrying waste is poultry farming. Concerning Brazil, chicken meat per capita consumption reaches 45 kg, and the annual output is approximately 14 million tons[1]. Feather keratin is one of the main valuable products to extract from poultry waste. Feathers are a residue that is slowly degraded in the environment, and their composition is approximately 90% of keratin, a fibrous and insoluble protein that gives recalcitrance[2] and when properly processed, can be converted into products with commercial value, such as food supplements, bio plastics, or textile fibers[3,4]. Keratinases are a type of hydrolytic specific enzymes that can catalyze keratin degradation and are secreted by different types of microorganisms found in soil, water, and various keratin-rich sources[5]. Given the need for innovative and sustainable solutions for the use of feathers from industrially farmed birds and the need to offer sustainable inputs for the textile industry, this research proposes the use of microorganisms that produce keratinases to degrade the structure of feathers and reuse them in the formation of fibers with textile application. To achieve this objective, cat hair was buried in a soil microcosm, and after 60 days, soil samples were transferred to culture media containing chicken feathers or cat hair as the main carbon source, an enrichment medium for keratinolytic microorganisms. Incubation occurred for 14 days under constant agitation, followed by isolation of the microorganisms. Initially, the selection of 10 microorganisms isolated from soils with feathers that could degrade this substrate was carried out. These isolates were grown in a medium containing chicken feathers as the main carbon and nitrogen source. After cultivation, the residual feathers were weighed, the concentration of proteins in the medium on different days was measured, and the proteolytic profile was analyzed. Preliminary results showed that of the ten strains, three showed more than 60% feather degradation. The dosages of soluble protein in the medium, following the assays of the enzymatic activity of the medium, showed good production of β-keratin degrading enzymes. Morphological analysis of the isolated strains indicated that they are gram-positive spore-forming bacteria. Optimizing the pH of the medium containing feathers showed that there is greater degradation of keratin in alkaline or slightly alkaline media.

2 MATERIAL & METHODS

To isolate keratinolytic microorganisms, cat hair (composed mainly of keratin) was buried in a microcosm containing soil cultivated with the ornamental plant *Capsicum annuum.* After 60 days, 1g soil samples were removed and transferred to 100mL culture media containing chicken feathers or cat hair as the primary carbon source, an enrichment medium for keratinolytic microorganisms. Incubation took place for 14 days under constant agitation (150 rpm), followed by the isolation of microorganisms through dilution and plating in a solid Luria Bertani (LB) medium. Initially, ten microorganisms were isolated with capable of degrading feather substrate. These isolates were cultivated in chicken feather medium. After cultivation for 7 days at room temperature (28ºC) and under constant agitation (180 rpm), the residual feathers were weighed. The supernatant was harvested to analyze the concentration of proteins using the Lowry protocol, the enzymatic activity (keratinases and peptidases) was quantified according to Mazotto et al. 2022[6], and the proteolytic profile was visualized by zymography.

3 RESULTS & DISCUSSION

In total, 10 strains of microorganisms were isolated and coded as PGSP for samples enriched in the medium with soil and PSP for samples enriched in the medium with soil and feathers. Preliminary results showed that, from ten strains, four degraded more than 60% of the feathers. They were identified as Gram-positive bacteria and probably belong to the Bacillus genre according to morphotintorial characteristics and MALDI-ToF analysis, which agrees with what is already known in the literature[6]. The feather degradation was estimated by weight loss and by the soluble protein concentration in the supernatant on different

days in order to observe the kinetics and degradation pattern of each isolated strain. The expression of extracellular peptidases and keratinases observed by soluble protein concentration assay shows the action of the enzymes on feathers (Figure 1). Also, the enzymatic activity assays using crushed feather (Figure 2A) and keratin azure (Figure 2B) showed good production of β-keratin degrading enzymes. Feather degradation is a complex mechanism that involves peptidases, keratinases, and reduction agents, resulting in keratin fragments with diverse molecular masses[2]. Different pH values were analyzed to improve the feather degradation by PSP 2.1, which proved to be the best strain. Optimization of the medium pH showed more significant keratin degradation in alkaline or slightly alkaline media (pH 8.5) (Figure 3).

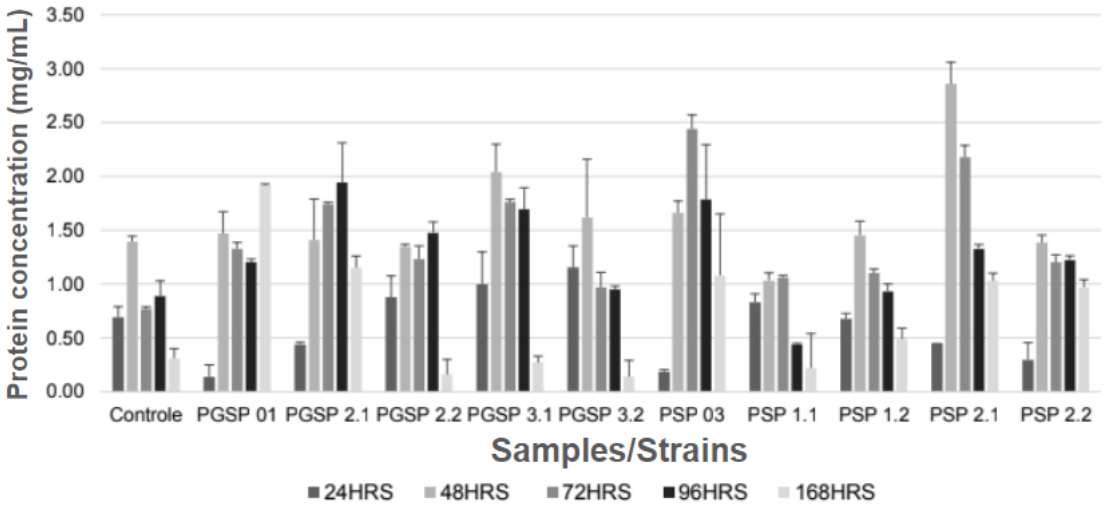

Figure 1. Comparison of protein concentration on different days in the supernatant media of bacteria isolated from soil cultivated in feather medium.

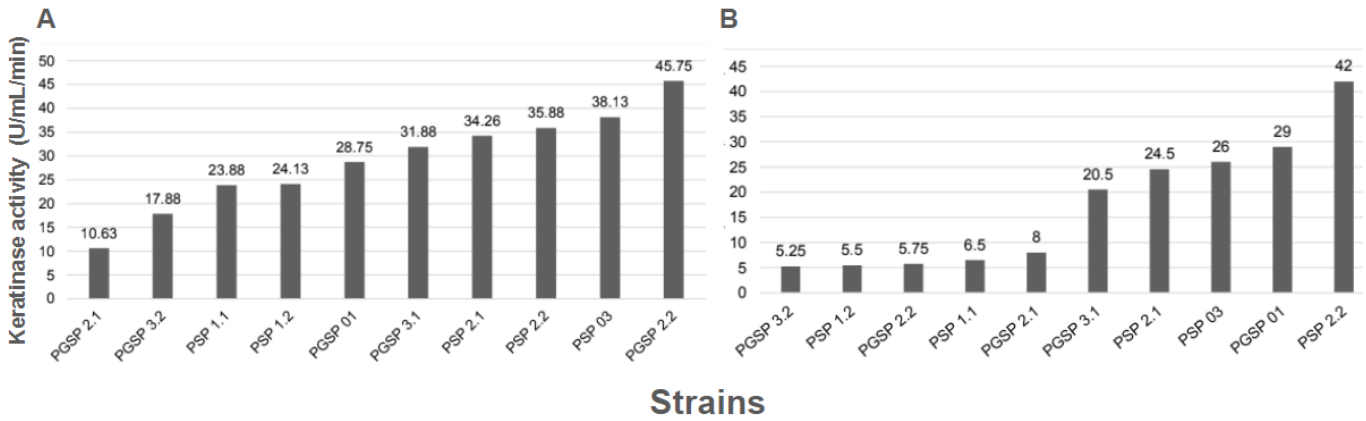

Figure 2. Quantification of enzyme keratinase in the culture supernatant of soil isolates after 7 days of cultivation in medium containing chicken feathers. A: α-keratinase; B: β-keratinase

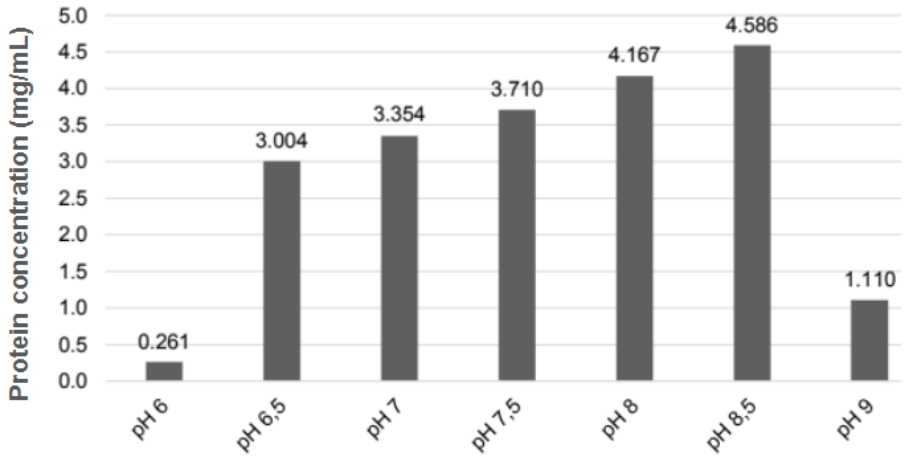

Figure 3. Protein concentration in the culture supernatant at different pHs.

4 CONCLUSION

The isolation of keratinolytic microorganisms from soils containing keratin and enrichment in feather or hair medium before plating led to obtaining relatively few strains; however, all of them produced peptidases with efficient feather degradation. The microcosm of soil with feathers allowed the isolation of 10 microorganisms capable of degrading keratinous substrates. Of these ten isolates, PSP 2.1 stood out for the high degradation of feathers in just 48h. Further studies aim to characterize the keratin fragments released during fermentation and optimize the keratinase production process.

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