Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 27, No. (3–4) : 2025 : 268-276
© EM International
ISSN-0972-3005

DOI No.: http://doi.org/10.53550/AJMBES.2025.v27i03-04.020
Journal Home Page: www.envirobiotechjournals.com

ENHANCED PRODUCTION AND PURIFICATION OF KERATINASE FROM *PAENIBACILLUS KOREENSIS* YC 300

SNEHA M.J., SUNEETHA P. AND C.S. KARIGAR*

Department of Biochemistry, Jnanabharathi Campus, Bangalore University, Bengaluru 560 056, Karnataka, India

(Received 7 April, 2025; Accepted 20 May, 2025)

Key words: *Keratinase, SmF, Purification, CFW remediation*

Abstract– Chicken feather waste is a high-protein byproduct of the poultry industry and a significant environmental concern due to its slow degradation. The current investigation is focused on the production and purification of keratinase from *Paenibacillus koreensis YC 300*. The maximum keratinase activity was observed after culturing *P. koreensis* with 1% inoculum for 5 days in the neutral medium (pH 7) at 45 °C. The keratinase was purified with 4% yield, specific activity of 320U/mg of protein, and a molecular mass of 67kDa. Keratinase and CFW degradation production were more efficient in submerged fermentation (SmF) than in the solid-state fermentation (SSF) process. The keratinase from fermentation showed optimal activity at pH 8 and 55°C. The structural changes owing to the degradation of CFW feathers were revealed by Scanning Electron Microscopy analysis. This study demonstrates that *P. koreensis* can influence CFW management, thereby offering an eco-friendly approach to the remediation of keratin-rich waste.

INTRODUCTION

A variety of domestic waste are enormously dumped into the environment causing a harsh and serious solid waste disposal problem. The extent of the natural accumulation of domestic wastes reflects a serious cause of toxic compounds and public health threats. Amongst the most complex biological materials is keratin-biomass, which comprises of skin appendages, hair, nails, tortoiseshells, horns, beaks, claws, and feathers (Wang *et al*., 2016). The animal meat manufacturing process generates millions of tonnes of keratinous materials and by-products (Li *et al*., 2020) Ivanovic *et al*., 2023). The quantity of meat produced in 2020 was about 100.5 million tonnes and more than 4.7 million tonnes of poultry feathers around the world (Pandey *et al*., 2019). On a yearly basis it is projected that more than 24.8 billion chickens will be produced yearly by 2030 reaching 37.0 billion in 2050 (United Nations of Food and Agriculture Organization (Alexandratos *et al*., 2012).

Keratin is considered as the third most abundant polymer in nature, following cellulose and chitin. Chicken feathers are composed of 91% protein (keratin), 1% lipids, and 8% of water. Keratin is an insoluble scleroprotein highly resistant to physical, and chemical activities (Femi-Ola *et al*., 2015) owing to the presence of several disulphide (S-S) cross-linkages (Kornillowicz-Kowalska *et al*.,1997). The keratinolytic microbes can degrade keratin by secreting keratinase (Lange *et al*., 2016; Jin *et al*., 2017). The biodegradation of keratininvolves sulfitolysis and proteolysis (Li *et al*., 2019). Sulfitolysis is the breakdown of disulfide bridges between the polypeptide chains of keratin (Callegaro *et al*., 2019) which causes the amino acids in the beta-sheet of keratin to shift conformation, resulting in new hydrolytic sites for keratinases (Tamreihao *et al*., 2019). Other methods in practice for keratin breakdown include physical, chemical, and biological processes (Shavandi *et al*., 2017). Some of these treatments require high temperature and pressure which destroy heat sensitive amino acids (like; tryptophan, lysine, and methionine) and generate significant amount of sulphur and ammonia gases (De Oliveira Martinez *et al*., 2020; Li *et al*., 2020). Therefore, microbial degradation

(Senior Professor)

strategy represents a promising alternative eco-friendly technology for recycling keratinous wastes (Ghasemi *et al*., 2012).

Generally, bacteria, fungi, and actinomycetes participate in keratin degradation. However, bacteria represent most abundant keratin-degraders, followed by fungi and actinomycetes (Wronska *et al*., 2016). Keratinolytic microbes are widely dispersed in nature and have been isolated from a variety of sources, including decomposing feathers (NagalSwetlana *et al*., 2009), penguin feathers (Pereira *et al*., 2014), poultry waste digester (Williams *et al*., 1990), and slaughter house polluted water (Fakhfakh-Zourai *et al*., 2010). Among potent keratin degrading bacteria are gram-positive and belong to the genus *Bacillus* species(Yong *et al*., 2020) including *B. halotolerans, B. cereus, B. licheniformis, B. subtilis* (Cedrola *et al*., 2012); *actinobacteria viz., Streptomyces fradiae, Nacardiopsis halotolarns, Amycolatopsis keratiniphila,* and fungi including *Aspergillus flavus, Trichophyton* sp., *Chrysosporiumindicum, Purpureocillium lilacinum* (Gradisar *et al*., 2005) have been reported and registered in literature with exceptional keratinolytic activity. Keratinases are mostly extracellular enzymes secreted into the culture medium, but cell-bound and intracellular enzymes have also been discovered (Singh *et al*., 2015).

Although many keratinolytic enzymes have been isolated over the years, the precise mechanism of keratin biodegradation remains to be clearly understood (Gonzalez *et al*., 2020; Kang *et al*., 2020). Mechanistically, keratin biodegradation is thought to involve adsorption of enzyme to the macromolecular surface followed by the catalytic action through the reduction of disulphide bonds(sulfitolysis) and disruption of the peptide chain (proteolysis) . This study attempts to characterize athermotolerant keratinase and optimize its production by fermentation, purification, and biochemical characterization.

MATERIALS AND METHODS

Keratinase from *Paenibacillus koreensis YC 300*

A keratinase-producing strain was isolated from chicken feather waste. The enzyme was extracted from *P. koreensis* cultured in feather meal broth (FMB: g/100ml: 0.05 NaCl, 0.04 KH_2PO_4, 0.03 K_2HPO_4, 1 chicken feather) and incubated at 45 °C, 100 rpm for 5 days. After centrifugation (10,000 rpm, 10 min, 4°C), the supernatant was used for keratinase assay (Sangali *et al*., 2000). Activity was measured using azocasein as substrate at 55 °C and pH 8.0, with absorbance read at 440 nm. One unit of activity corresponded to an absorbance change of 0.01 in 10 minutes. Protein content was determined by the Folin-Ciocalteu method (Lowry *et al*., 1951) and free amino acids by the ninhydrin method (Moore *et al*., 1948).

Optimization of Keratinase Production

The growth of *P. koreensis* was evaluated under varying conditions. pH levels (5-9), temperatures (35-55 °C), and different nitrogen (ammonium sulphate, urea, yeast extract, peptone) and carbon sources (glucose, sucrose, mannose) were tested in 100 mL of FMB. Feather concentrations (0.5-2%) and inoculum sizes (1-5%) were also varied to assess their effects on growth. Finally, keratinase production was monitored at different time intervals (24-144 hrs) using optimized conditions (Barman *et al*., 2017).

Production of keratinase in Solid State Fermentation (SSF) and Submerged Fermentation (SmF)

Solid State Fermentation (SSF)

SSF was carried out by inoculating 1 ml of overnight culture into 80 ml of FMB (40% moisture) containing 2 g of chicken feathers. The setup was incubated at 45°C without agitation for 5 days. Keratinase activity, free amino acids, and feather degradation were measured every 5 days (Nurkhasanah *et al*., 2019).

Moisture = Volume of medium (ml)/ chicken feathers (g)× 100%

Submerged Fermentation (SmF)

In SmF, 1 g of feather was added to FMB, sterilized, and inoculated with 1 ml of overnight culture. The culture was incubated at 45°C, 100 rpm for 15 days. After day 15, fresh FMB was added, and fermentation continued to day 30 with samples taken every 5 days for analysis (Mazotto *et al*., 2013).

Scanning electron microscopy (SEM) of chicken feathers

The feathers after fermentation by SSF and SmF were collected, washed with distilled water, dried at room temperature, and subjected to SEM to examine alterations in morphological features (Chaturvedi *et al*., 2014).

Fourier Transform Infrared Spectroscopy (FTIR) analysis of chicken feathers

The changes in functional groups occurring during degradative fermentations by SmF and SSF were analysed by FTIR analysis (Tesfaye *et al*., 2017).

Purification of Keratinase

The fermentation broth was centrifuged at 10,000 rpm for 10 minutes at 4 °C, and the resulting supernatant was used for keratinase purification. Initial protein precipitation was carried out using 0-80% ammonium sulfate under constant stirring at 4 °C for 1 hour, followed by overnight refrigeration. The precipitated protein was then collected by cold centrifugation and dissolved in 0.1 M Tris buffer (pH 8.0). The solution was dialyzed overnight at 0-4 °C against 10 mMTris buffer (pH 8.0) to remove excess salts. The dialyzed sample was subjected to ion exchange chromatography using a DEAE-cellulose column pre-equilibrated with 50 mM Tris buffer (pH 8.0), and elution was performed using a 0.5 M NaCl gradient at a flow rate of 24 ml/h. Active fractions showing keratinase activity were pooled and purified through gel filtration chromatography on a Sephacryl S-200 column, eluted with 50 mM Tris buffer (pH 8.0) at 10 ml/hr. The collected fractions were analyzed for both keratinase activity and protein concentration (Ramalingum *et al*., 1970).

BiochemicalCharacterization of Enzyme

Determination of molecular weight of keratinase by SDS-PAGE

Keratinase-active fractions from gel filtration were pooled, lyophilized, and analyzed by SDS-PAGE (12% resolving, 5% stacking gel) (Gupta *et al*., 2015). Molecular weight was determined by comparing Rf values with standard proteins.

Effect of pH and temperature

Studies on the effect of pH and temperature on keratinase were performed as described before using 50 mM Acetate buffer (pH 4.5- 5.5), 50 mM Phosphate buffer (pH 6.0 - 7.0), 50mM Tris buffer (pH 7.5 - 9.5) and temperature range of 25 °C- 70 °C. The experiments on the effect of pH and temperature on keratinase stability were carried out by incubating the keratinase at pH (4.5- 9.5) and temperature (25 - 70°C). The standard enzyme assay then determined the enzyme activities (Verma *et al*., 2017).

Table 1. Properties of the culture broth after 5 days of keratinase production by *P. koreensis*(mean ± SD, n=3).

Keratinase	Units
Keratinase activity	1.8U/ml ± 0.2517
Protein	0.096 mg/ml ± 0.01930
Free Amino acids	0.013435 mg/ml ± 0.0100
Specific activity	18.75U/mg ±1.600

Effect of Enzyme Inhibitors

The inhibitor effect was assessed by incubating the keratinase with 1- 10mM of protease inhibitor; phenylmethanesulphonyl fluoride(PMSF), ethylenediaminetetraacetate (EDTA), and iodoacetamide (IAA) at 30 °C for 10 min (Embaby *et al.,* 2015).

Determination of K_m and V_{max}

Keratinase kinetic parameters were measured at different substrate concentrations (2- 10 mg) under optimum assay conditions, Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) values were determined with Line weaver-Burk plots (Israel-Roming *et al*., 2014).

Statistical Analysis

All the experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD) using GraphPad Prism version 5.

RESULTS

P. koreensis was isolated from CFW and cultured in FMB. After 5 days, keratinase was detected in the culture supernatant. The following results were observed:

Optimization of growth conditions for keratinase production

Keratinase production was optimized under different conditions. The highest enzyme activity (98.29 U/mg ± 1.105) and free amino acid content (8.09 mg/ml± 0.130) were observed at pH 7.0. The optimal temperature was 45 °C, with keratinase activity of (74.17 U/mg ± 3.66) and amino acid release of (10.29mg/mL ± 0.9552). Peptone was the most effective nitrogen source, yielding (11.39 U/mg ± 1.631) of keratinase and (0.37 mg/ml ± 0.031) of amino acids. Glucose supported the highest keratinase production (8.18 U ± 0.556). A 1% feather concentration gave maximum enzyme activity (28.05 U/mg ± 2.55), while 2% yielded the most

amino acids (0.38 mg/ml ± 0.059). An inoculum size of 5% was ideal, producing (24.75 U/mg ± 0.6502) keratinase and (1.04 mg/ml ± 0.096) amino acids. The best incubation time was five days, resulting in (59.14 U/mg ± 0.2452) of keratinase and (7.702 mg/ml ± 0.5474) of free amino acids.

Comparative study on keratinase production and feather degradation by Solid State Fermentation (SSF) and Submerged Fermentation (SmF)

Keratinase and free amino acid production varied between SSF and SmF. Under SSF, maximum levels were reached by day 5 (9.527U/mg± 0.4446 keratinase; 0.03 mg/ml± 0.0010 amino acids), while in SmF, peak production occurred at day 25 (34.21U/mg ± 0.744 keratinase; 0.015 ± 0.0004163 mg/mlamino acids).

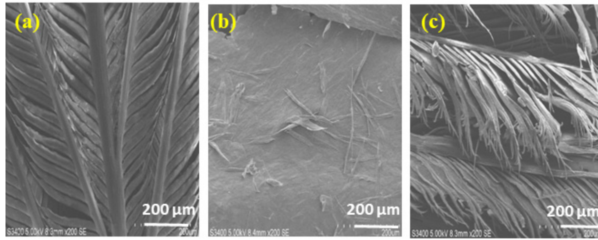

Fig. 1. SEM micrographs: Control (a) ; partially degraded feather by SmF (b); and partially degraded feather (c) by SSF.

SEM analysis

P. koreensis treatment caused noticeable feather degradation. Unlike the intact structure in untreated feathers (Figure 1), those under SSF and SmF showed partial breakdown of the rachis, barbs, and barbules.

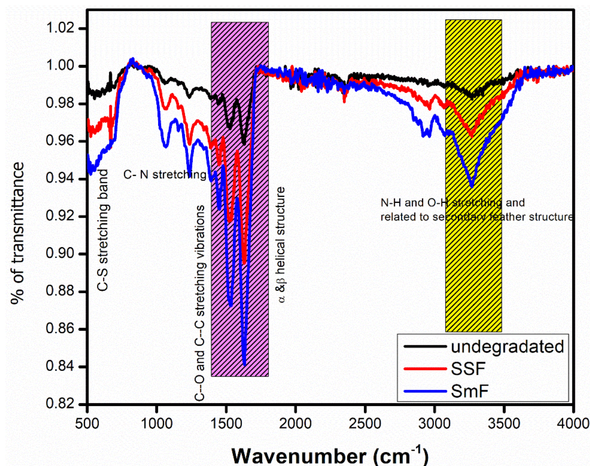

Fig. 2. FTIR analysis of feather degraded under SSF and SmF.

FTIR analysis

FTIR analysis (Figure 2) of feathers treated with *P. koreensis* showed reduced disulfide bonds and changes in protein secondary structure compared to untreated feathers.

Purification of Keratinase

Keratinase from *P. koreensis* was purified using 80% ammonium sulfate precipitation, DEAE-Sepharose ion exchange, and Sephacryl S-200 gel filtration. Peak activity was observed in DEAE fractions 23–30 and Sephacryl fractions 11-17 (Figures 3 & 4). The process achieved a 4% yield and 25-fold purification (Table 2).

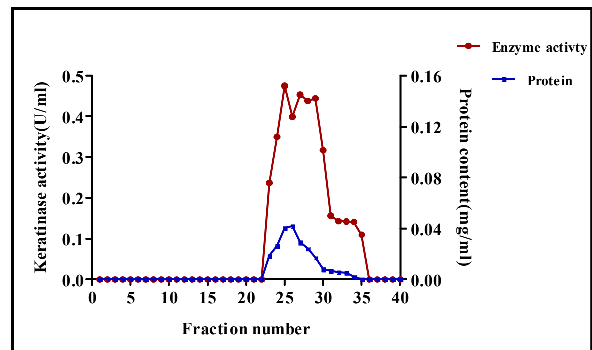

Fig. 3. Elution profile of keratinase from *P. koreensis* by DEAE cellulose chromatography.

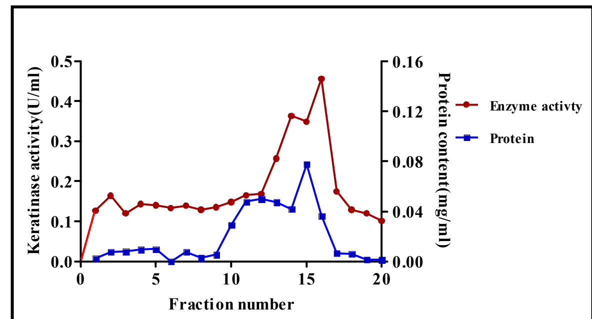

Fig. 4. Elution profile of keratinase from *P. koreensis* by Gel filtration column chromatography.

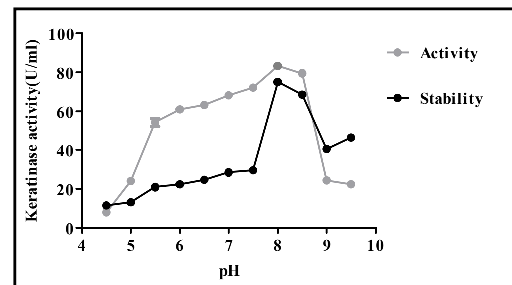

Fig. 5. Effect of pH on the activity and stability of purified keratinase. Data are mean ± SD (n = 3)

Table 2. Purification of *P. koreensis* keratinase.

Purification Steps	Volume (ml)	Total activity (U/ml)	Total protein (mg/ml)	Specific activity (U/mg)	Fold purification	% Yield
Crude	100	96.17	0.987	99.45	1	100
Ammonium sulfatefractionation (0-80%)	15	82.11	0.761	107.05	1.303	76.70
DEAE Ion Exchange Chromatography	28	22.8	0.104	219.23	9.61	10.400
Sephacryl S-200 gelfiltration Chromatography	8	12.8	0.0040	320	25	4

Keratinase biochemical properties

SDS-PAGE revealed the molecular weight of purified keratinase from *P. koreensis* YC300 as 67 kDa (Figure 7). The enzyme showed optimal activity and stability at 55°C (Figure 6) and pH 8.0 (Figure 5), but lost activity at higher temperatures. PMSF strongly inhibited keratinase, indicating it's a serine protease (Table 3). Kinetic studies were performed at pH 8.0 and 55°C (Figure 8).

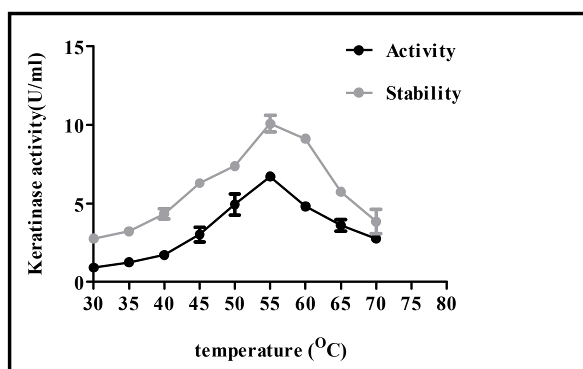

Fig. 6. Effect of temperature on the activity and stability of purified keratinase. (Data are mean ± SD; n = 3)

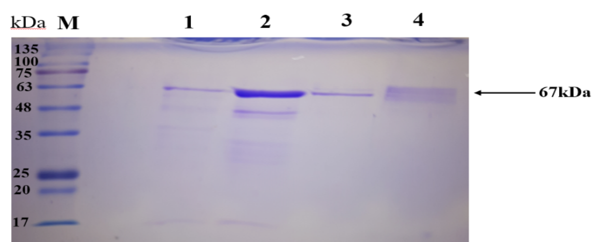

Fig. 7. Determination of molecular weight of *P. koreensis* keratinase by SDS-PAGE. Lane M; protein ladder, Lane 1; Crude, Lane 2; Ammonium sulphate (80%) fractionation, Lane 3; DEAE column fraction and Lane 4; Sephacryl S-200 Gel filtration column fraction

Table 3. Effect of inhibitors on keratinase from *P. koreensis*

Inhibitors	% inhibition	
	10mM	5mM
PMSF	81	55
EDTA	7	2
IAA	28	18

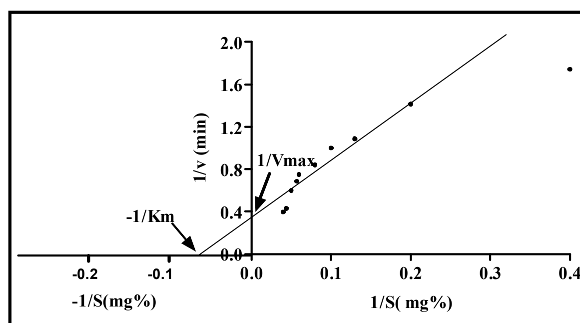

Fig. 8. Lineweaver-Burk plot for keratinase (azocasein as a substrate) from *P. koreensis*

DISCUSSION

Microorganisms are the resources of variety of degradative enzymes including keratinases (Kamal *et al*., 2017). The primary step involved in the commercial enzyme production is to find a suitable microorganism that can produce enzymes in high concentrations. This is followed by optimization of the process parameters (media, pH, temperature, etc.). Screening of the soil from poultry farms resulted in isolation of a feather-degrading bacterium. The bacterium was identified as *Paenibacillus koreensis YC300* based on 16S rRNA gene sequencing and phylogenetic analysis (NCBIAccession number: PP059639). The bacterium belongs to *Paenibacillus* genus whichis equivalent to *Bacillus* (Paul *et al*., 2014). The literature survey resulted in a report on the degradation of CFW by *Paenibacillus woosongensis TKB2* degrading 87 % CFW (0.8 %, w/v) in 2 days (Sivakuar *et al*., 2012). This is the first communication reporting the degradation of feathers by *P. koreensis*. The optimum culture conditions for keratinase production have been investigated and established for *P. koreensis*. The maximum activity observed at pH 8 and 55°C indicates that keratinase is alkaliphilic and thermophilic (Agrahari *et al*., 2010; Fuke *et al*., 2018).

The pH is known to influence keratinase production by involving in the transfer of various nutrients in and out of the microorganism cell membrane (Gaurav *et al*., 2016). The bacterium *P. koreensis* showed maximum keratinase activityat pH7. Keratinases from most microorganisms are best active in the range of neutral and alkaline pH (Riessen *et al*., 2001). The enzyme required for industrial purpose is expected to retain its activity over a wide range of pH. Temperature is another important parameter that influences production by regulating its biosynthesis and energy metabolism. The optimum temperature for keratinase production and amino acids was found to be 45°C. Many keratinolytic bacteria often show profuse activity at higher temperature from 30°C - 55°C. An optimal temperature of 70 °C has been reported for *Thermoanaerobacter* (Nam GaeWon *et al.,* 2002) and *Fervidobacterium* spp. (Ghaffar *et al*., 2018). In our study chicken feather served as the only source of carbon and nitrogen for keratinase production. The experiments showed that incorporating additional simple sugars like glucose into the culture medium lead to decreased keratinase production; in contrast inclusion of complex carbohydrates like starch showed elevated keratinase production (Cai *et al*., 2008). Among the various substrates incorporation of feather concentration of 1.0% has shown the highest keratinase and amino acid production (Ni *et al*., 2011) and 2% (Subathra Devi *et al.,* 2018). The feather can act as substrate and inducer or inhibitor for keratinase production. Inoculum size of 2.0 % showed enhanced keratinase and amino acid production. A study reported the optimum production of keratinase at 2% inoculum for *Bacillus cereus* (Sivakumar *et al*., 2013). Another study found that increasing the inoculum concentration to 4% resulted in an increase in keratinase synthesis by *B. cereus* (Subramaniyam *et al*., 2012). The accumulation of keratinase and amino acids in culture broth occurred from 24 h to 144 hr.

Comparative analysis showed that *P. koreensis* in SmF led to higher keratinase activity and 68% feather degradation, compared to 33% in SSF. Unlike previous reports favoring SSF for better degradation, this study found SmF more effective, likely due to its aqueous nature supporting enzyme secretion. SEM analysis confirmed distinct morphological changes in feathers under both conditions. The SEM results demonstrated that the bacterium was able to hydrolyse soft structures (outer vane, inner vane, and parallel barbs) completely in SSF whereas in SmF hard structures (feather tip and rachis) were degraded partially. The FTIR spectral observations displayed peaks at 3200-3400 cm^{-1} corresponding to the OH and SH, followed by peaks at 1300,1400, 1500 and 1600 cm^{-1} corresponding indicate C-N, C-O, C=O, and α, β helical structures in proteins. The degraded feathers under SSF and SmF showed peaks of high resolution (red & blue). Among SmF and SSF, SmFshowed efficient degradation as indicated by broad and intense peaks at 3400 and 3300 cm^{-1} free N-H and O-H stretching groups. A sharp peak at 1600, and 1560 cm^{-1} reveals the presence of keto and amide groups. The 1350-1400 cm^{-1} spectral peaks correspond to the C-C and C-O bonds. A weak C-S stretching band is observed at the lower wavenumber region (600). Some small peaks correspond to the binding modes of amide groups. The above results suggest that feather degradation under SmF is more than SSF. The results obtained in the current study showed more efficiency of feather degradation (Uttangi *et al*., 2018).

The keratinase has been purified sequentially by ammonium sulphate precipitation, dialysis, ion exchange and gel filtration procedures (Table 2). Similar purification strategy has been adopted for enzyme purification from *Bacillus subtilis* KD-N2 (Jaouadi *et al*., 2016), *Bacillus subtilis* (Kainoor *et al*., 2010), *Bacillus tequilensis* strain Q7 (Lee *et al.,* 2002). The molecular weight of keratinase is established to be 67kDa by SDS-PAGE analysis. However, previous reports hint at presence of varying keratinase mass in other organisms; *Bacillus sp. JB 99* (66kDa) (Ghosh *et al*., 2008), *Streptomyces albidoflavus* (18 kDa), *Bacillus sp. SCB3*(134kDa) (Qiu *et al*., 2020), *B. cereus DCUW* (80kDa) (Manachini *et al*., 1988), *Bacillus tequilensis* strain *Q7* (28.3 kDa) (Singh *et al*., 2001), *Bacillus subtilis* (40kDa) and a keratinase between 26 and 130 kDa (Tian *et al*., 2019). The keratinase from *P. koreensis YC 300* is inhibited by PMSF, hence it belongs to a serine protease family. Several reports have shown that metalloprotease inhibitors influence serine proteases slightly (Duffeck *et al*., 2020), and keratinase from gram-positive bacteria are mostly serine proteases. The kinetic studies on enzymes aim to measure the affinity with which the enzyme binds to substrate and the turnover rate. There is a report that shows a sigmoidal curve for varied concentrations of substrate and also enzyme had a high Km value. Thus, this attempt achievedthe successful optimization and purification of keratinase from *P.*

koreensis that marks a significant step towards development of a sustainable biotechnological processes.

CONCLUSION

In conclusion, this study successfully optimized the physical parameters required for keratinase production by *P. koreensis*, achieving optimal enzyme activity and efficiency under specific pH and temperature conditions. The identification of *P. koreensis* as a novel keratinase producer highlights the vast potential of natural microbial sources for industrial applications enabling the development of sustainable and eco-friendly solutions. This study contributes significantly to the expanding field of keratinase research, identifying *P. koreensis* as a promising resource for industrial applications.

ACKNOWLEDGEMENT

The authors thank the Department of Biochemistry, Bangalore University, for research support, and Prof. H. Manjunatha for his valuable assistance. Appreciation is also extended to Nandisha PS, Vinay G, and Prof. Shivaraj from the Department of Chemistry for their support.

Conflict of Interest

There is no conflict of interest between the authors, all authors contributed directly to the article.

REFERENCES

Agrahari, S. and Wadhwa, N. 2010. Degradation of chicken feather a poultry waste product by keratinolytic bacteria isolated from dumping site at Ghazipur poultry processing plant. *Int J Poult Sci.* 9(5): 482-489.

Alexandratos, N. and Bruinsma, J. 2012. World agriculture towards 2030/2050: the 2012 revision.

Barman, N.C., Zohora, F.T., Das, K.C., Mowla, M.G., Banu, N.A., Salimullah, M. and Hashem, A. 2017. Production, partial optimization and characterization of keratinase enzyme by *Arthrobacter* sp. NFH5 isolated from soil samples. *Amb Express.* 7(1): 181.

Cai, C.G., Lou, B.G. and Zheng, X.D. 2008. Keratinase production and keratin degradation by a mutant strain of Bacillus subtilis. *Journal of Zhejiang University Science B.* 9(1): 60-67.

Callegaro, K., Brandelli, A. and Daroit, D.J. 2019. Beyond plucking: feathers bioprocessing into valuable protein hydrolysates. *Waste Management.* 95: 399-415.

Cedrola, S.M.L., de Melo, A.C.N., Mazotto, A.M., Lins, U., Zingali, R.B., Rosado, A.S. and Vermelho, A. B. 2012. Keratinases and sulfide from *Bacillus subtilis* SLC to recycle feather waste. *World Journal of Microbiology and Biotechnology*. 28: 1259-1269.

Chaturvedi, V., Bhange, K., Bhatt, R. and Verma, P. 2014. Production of kertinases using chicken feathers as substrate by a novel multifunctional strain of *Pseudomonas stutzeri* and its dehairing application. *Biocatalysis and Agricultural Biotechnology*. 3(2): 167-174.

De Oliveira Martinez, J. P., Cai, G., Nachtschatt, M., Navone, L., Zhang, Z., Robins, K. and Speight, R. 2020. Challenges and opportunities in identifying and characterising keratinases for value-added peptide production. *Catalysts*. 10(2): 184.

Duffeck, C.E., de Menezes, C.L.A., Boscolo, M., da Silva, R., Gomes, E. and da Silva, R.R. 2020. Citrobacterdiversus-derived keratinases and their potential application as detergent-compatible cloth-cleaning agents. *Brazilian Journal of Microbiology.* 51: 969-977.

Embaby, A.M., Marey, H.S. and Hussein, A. 2015. A statistical-mathematical model to optimize chicken feather waste bioconversion via *Bacillus licheniformis* SHG10: a low cost effective and ecologically safe approach. *Journal of Bioprocessing &Biotechniques*. 5(6): 1.

Fakhfakh-Zouari, N., Haddar, A., Hmidet, N., Frikha, F. and Nasri, M. 2010. Application of statistical experimental design for optimization of keratinases production by *Bacillus pumilus* A1 grown on chicken feather and some biochemical properties. *Process Biochemistry.* 45(5): 617-626.

Femi-Ola, T.O., Akinbobola, O.S. and Oluwaniyi, T.T. 2015. Isolation and characterization of feather degrading bacteria from poultry soil. *Agriculture and Biology Journal of North America*. 6(5): 146-154.

Fuke, P., Anshuman, A., Khardenavis, A., Hermant, J. and Purohit, H. J. 2018. Recent trends in bacterial keratinases. *Recent Advances in Biotechnology.* 1: 33-52.

Gaurav Sanghvi, G.S., Harshit Patel, H.P., DevendraVaishnav, D.V., TejasOza, T.O., Gaurav Dave, G.D., Prashant Kunjadia, P.K. and NavinSheth, N.S. 2016. A novel alkaline keratinase from *Bacillus subtilis* DP1 with potential utility in cosmetic formulation. *International Journal of Biological Macromolecules*. 87: 256-262.

Ghaffar, I., Imtiaz, A., Hussain, A., Javid, A., Jabeen, F., Akmal, M. and Qazi, J.I. 2018. Microbial production and industrial applications of keratinases: an overview. *International Microbiology.* 21: 163-174.

Ghasemi, Y., Shahbazi, M., Rasoul-Amini, S., Kargar, M., Safari, A., Kazemi, A. and Montazeri-Najafabady, N. 2012. Identification and characterization of feather-degrading bacteria from keratin-rich wastes. *Annals of MIcrobiology.* 62: 737-744.

Ghosh, A., Chakrabarti, K. and Chattopadhyay, D. 2008.

Degradation of raw feather by a novel high molecular weight extracellular protease from newly isolated *Bacillus cereus* DCUW. *Journal of Industrial Microbiology and Biotechnology*. 35(8): 825-834.

González, V., Vargas-Straube, M.J., Beys-da-Silva, W.O., Santi, L., Valencia, P., Beltrametti, F. and Cámara, B. 2020. Enzyme bioprospection of marine-derived actinobacteria from the chilean coast and new insight in the mechanism of keratin degradation in *Streptomyces* sp. G11C. *Marine Drugs*. 18(11): 537.

Gradis˜ar, H., Friedrich, J., Krizaj, I. and Jerala, R. 2005. Similarities and specificities of fungal keratinolytic proteases: comparison of keratinases of *Paecilomycesmarquandii* and *Doratomycesmicrosporus* to some known proteases. *Applied and Environmental Microbiology.* 71(7): 3420-3426.

Gupta, S., Nigam, A. and Singh, R. 2015. Purification and characterization of a *Bacillus subtilis* keratinase and its prospective application in feed industry. *Acta Biologica Szegediensis*. 59(2): 197-204.

Ghosh, A., Chakrabarti, K. and Chattopadhyay, D. 2008. Degradation of raw feather by a novel high molecular weight extracellular protease from newly isolated Bacillus cereus DCUW. *Journal of Industrial Microbiology and Biotechnology*. 35(8): 825-834.

Ivanoviæ, N., Iliæ, T., Æiriæ, M.Z., Todoroviæ, V., Djurièiæ, I. and Dabetiæ, N. 2023. Agri-food by-products as a source of sustainable ingredients for the production of functional foods and nutraceuticals. *Archives of Pharmacy.* 73(3): 190-204.

Israel-Roming, F., Cornea, P., Gherghina, E., Lutã, G. and Bãlan, D. 2014. Bacterial proteolytic enzymes tested on keratin and collagen based material. *Scientific Bulletin. Series F. Biotechnologies*. 18: 169-173.

Jaouadi, N.Z., Rekik, H., Elhoul, M. B., Rahem, F.Z., Hila, C.G., Aicha, H. S.B. and Jaouadi, B. 2015. A novel keratinase from *Bacillus* tequilensis strain Q7 with promising potential for the leather bating process. *International Journal of Biological Macromolecules.* 79: 952-964.

Jin, H.S., Park, S.Y., Kim, K., Lee, Y.J., Nam, G.W., Kang, N.J. and Lee, D.W. 2017. Development of a keratinase activity assay using recombinant chicken feather keratin substrates. *PloS One.* 12(2): e0172712.

Kainoor, P.S. and Naik, G.R. 2010. Production and characterization of feather degrading keratinase from *Bacillus* sp. *Indian Journal of Biotechnology*. 9: 384-390.

Kamal, S., Rehman, S. and Iqbal, H.M. 2017. Biotechnological valorization of proteases: from hyperproduction to industrial exploitation-a review. *Environmental Progress & Sustainable Energy.* 36(2): 511-522.

Kang, E., Jin, H.S., La, J.W., Sung, J. Y., Park, S.Y., Kim, W.C. and Lee, D.W. 2020. Identification of keratinases from Fervidobacteriumislandicum AW1 using dynamic gene expression profiling. *Microbial Biotechnology.* 13(2): 442-457.

Korni³³owicz-Kowalska, T. 1997. Studies on the decomposition of keratin wastes by saprotrophic microfungi. II. Sulphur and nitrogen balance. *Acta Mycologica.* 32(1): 81-93.

Lange, L., Huang, Y. and Busk, P.K. 2016. Microbial decomposition of keratin in nature-a new hypothesis of industrial relevance. *Applied Microbiology and Biotechnology*. 100(5): 2083-2096.

Lee, H., Suh, D.B., Hwang, J.H. and Suh, H.J. 2002. Characterization of a kerationlytic metalloprotease from Bacillus sp. SCB-3. *Applied Biochemistry and Biotechnology*. 97: 123-133.

Li, Q. 2019. Progress in microbial degradation of feather waste. *Frontiers in Microbiology*. 10: 2717.

Li, Z. W., Liang, S., Ke, Y., Deng, J.J., Zhang, M.S., Lu, D.L. and Luo, X.C. 2020. The feather degradation mechanisms of a new Streptomyces sp. isolate SCUT-3. *Communications Biology*. 3(1): 191.

Li, Z., Reimer, C., Picard, M., Mohanty, A. K. and Misra, M. 2020. Characterization of chicken feather biocarbon for use in sustainable biocomposites. *Frontiers in Materials*. 7: 3.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

Manachini, P.L., Fortina, M.G. and Parini, C. 1988. Thermostable alkaline protease produced by *Bacillus* thermoruber-a new species of Bacillus. *Applied Microbiology and Biotechnology*. 28: 409-413.

Mazotto, A.M., Couri, S., Damaso, M.C. and Vermelho, A.B. 2013. Degradation of feather waste by *Aspergillus niger* keratinases: comparison of submerged and solid-state fermentation. *International Biodeterioration & Biodegradation.* 85: 189-195.

Moore, S. and Stein, W.H. 1948. *Methods in Enzymol*. Academic Press, New York, 3: 468.

NagalSwetlana, N.S. and Jain, P.C. 2009. Feather degradation by strains of *Bacillus* isolated from decomposing feathers. *Brazillian Journal of Microbiology*. 4(1): 196-200

Nam GaeWon, N.G., Lee DongWoo, L.D., Lee HanSeoung, L.H., Lee NamJu, L.N., Kim ByoungChan, K.B., ChoeEunAh, C.E. and PyunYuRyang, P.Y. 2002. Native-feather degradation by *Fervidobacterium islandicum* AW-1, a newly isolated keratinase-producing thermophilic anaerobe. *Archives of Microbiology.* 178(6): 538-547.

Ni, H., Chen, Q.H., Chen, F., Fu, M.L., Dong, Y.C. and Cai, H.N. 2011. Improved keratinase production for feather degradation by *Bacillus licheniformis* ZJUEL31410 in submerged cultivation. *African Journal of Biotechnology.* 10(37): 7236-7244.

Nurkhasanah, U. 2019. Preliminary study on keratinase fermentation by *Bacillus* sp. MD24 under solid state fermentation. In: *IOP Conference Series: Earth and Environmental Science.* 276(1): 012016). IOP Publishing.

Pandey, S.C., Pande, V., Sati, D., Gangola, S., Kumar, S., Pandey, A. and Samant, M. 2019. Microbial keratinase: a tool for bioremediation of feather waste.

In: *Smart Bioremediation Technologies* (pp. 217-253). Academic Press.

Paul, T., Das, A., Mandal, A., Halder, S.K., DasMohapatra, P.K., Pati, B.R. and Mondal, K.C. 2014. Valorization of chicken feather waste for concomitant production of keratinase, oligopeptides and essential amino acids under submerged fermentation by *Paenibacillus woosongensis* TKB2. *Waste and Biomass Valorization.* 5: 575-584.

Pereira, J.Q., Lopes, F.C., Petry, M.V., da Costa Medina, L.F. and Brandelli, A. 2014. Isolation of three novel Antarctic psychrotolerant feather-degrading bacteria and partial purification of keratinolytic enzyme from *Lysobacter* sp. A03. *International Biodeterioration & Biodegradation*. 88: 1-7.

Qiu, J., Wilkens, C., Barrett, K., & Meyer, A. S. 2020. Microbial enzymes catalyzing keratin degradation: Classification, structure, function. *Biotechnology Advances*. 44: 107607.

Ramalingum, N., Bhagwat, P., Permaul, K. and Pillai, S. 2024. Production, characterization, and application of *Pseudomonas aeruginosa* S-04 keratinase for feather utilization. *Biomass Conversion and Biorefinery*. 14(10): 11683-11695.

Riessen, S. and Antranikian, G. 2001. Isolation of *Thermoanaerobacter keratinophilus* sp. nov., a novel thermophilic, anaerobic bacterium with keratinolytic activity. *Extremophiles.* 5, 399-408.

Sangali, S. and Brandelli, A. 2000. Isolation and characterization of a novel feather-degrading bacterial strain. *Applied biochemistry and Biotechnology*. 87: 17-24.

Sharma, I., Pranaw, K., Soni, H., Rawat, H.K. and Kango, N. 2022. Parametrically optimized feather degradation by *Bacillus velezensis* NCIM 5802 and delineation of keratin hydrolysis by multi-scale analysis for poultry waste management. *Scientific Reports.* 12(1): 17118.

Shavandi, A., Silva, T.H., Bekhit, A.A. and Bekhit, A.E.D.A. 2017. Keratin: dissolution, extraction and biomedical application. *Biomaterials Science.* 5(9): 1699-1735.

Singh, I. and Kushwaha, R.K.S. 2015. Keratinases and microbial degradation of keratin. *Adv Appl Sci Res*. 6(2): 74-82.

Singh, J., Vohra, R.M. and Sahoo, D.K. 2001. Purification and characterization of two extracellular alkaline proteases from a newly isolated obligate alkalophilic *Bacillus sphaericus. Journal of Industrial Microbiology and Biotechnology*. 26(6): 387-393.

Sivakumar, T., Shankar, T., Vijayabaskar, P. and Ramasubramanian, V. 2012. Optimization for keratinase enzyme production using *Bacillus thuringiensis* TS2. *Academic Journal of Plant Sciences*. 5(3): 102-109.

Sivakumar, T., Shankar, T., Thangapand, V. and Ramasubram, V. 2013. Optimization of cultural condition for keratinase production using Bacillus cereus TS1. *Insight Microbiol.* 3(1): 1-8.

Subathra Devi, C., Shankar, R., Kumar, S., Mohanasrinivasan, V. and Vaishnavi, B. 2018. Production of keratinase from a newly isolated feather degrading *Bacillus cereus* VITSDVM4 from poultry waste. *National Academy Science Letters.* 41: 307-311.

Subramaniyam, R. and Vimala, R. 2012. Solid state and submerged fermentation for the production of bioactive substances: a comparative study. *Int J Sci Nat.* 3(3): 480-486.

Tamreihao, K., Mukherjee, S., Khunjamayum, R., Devi, L.J., Asem, R.S. and Ningthoujam, D.S. 2019. Feather degradation by keratinolytic bacteria and biofertilizing potential for sustainable agricultural production. *Journal of Basic Microbiology.* 59(1): 4-13.

Tesfaye, T., Sithole, B., Ramjugernath, D. and Chunilall, V. 2017. Valorisation of chicken feathers: Characterisation of chemical properties. *Waste Management*. 68: 626-635.

Tian, J., Xu, Z., Long, X., Tian, Y. and Shi, B. 2019. High-expression keratinase by *Bacillus subtilis* SCK6 for enzymatic dehairing of goatskins. *International Journal of Biological Macromolecules*. 135: 119-126.

Uttangi, V. and Aruna, K. 2018. Optimization of production and partial characterization of keratinase produced by *Bacillus thuringiensis* strain Bt407 isolated from poultry soil. *Int J Curr Microbiol App Sci*. 7(4): 596-626.

Verma, A., Singh, H., Anwar, S., Chattopadhyay, A., Tiwari, K.K., Kaur, S. and Dhilon, G. S. 2017. Microbial keratinases: industrial enzymes with waste management potential. *Critical Reviews in Biotechnology*. 37(4): 476-491.

Wang, B., Yang, W., McKittrick, J. and Meyers, M. A. 2016. Keratin: Structure, mechanical properties, occurrence in biological organisms, and efforts at bioinspiration. *Progress in Materials Science*. 76: 229-318.

Williams, C.M., Richter, C.S., MacKenzie Jr, J.M. and Shih, J.C. 1990. Isolation, identification, and characterization of a feather-degrading bacterium. *Applied and Environmental Microbiology*. 56(6): 1509-1515.

Wroñska, I. and Cybulska, K. 2016. Presence of microorganisms at various stages of poultry wastes management. Part I. Keratinolytic microorganisms. *Journal of Ecological Engineering*, 17(5): 43-48.

Yong, B., Fei, X., Shao, H., Xu, P., Hu, Y., Ni, W. and Feng, H. 2020. Recombinant expression and biochemical characterization of a novel keratinase BsKER71 from feather degrading bacterium *Bacillus subtilis* S1-4. *AMB Express.* 10: 1-10.