

Production of microbial keratinase using a newly isolated strain of *Stenotrophomonas maltophilia* and its characterization and applications.

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Abstract

Biodegradation of keratin containing wastes viz. bird feathers, nails, hairs, animal wool is a low-cost, nutrient-rich biotechnological process that turns this plentiful waste into a low-cost, nutrient-rich substance. It is a sustainable green approach towards the solution of environmental threats created by these wastes. This study aimed to screen for potential keratinase-producing microorganisms and to optimize physicochemical parameters to produce keratinase and its characterization. Samples were collected from various poultry farms of Surat (India). Screening for keratinase-production was carried using a feather basal medium. Among these samples, 16 isolates showed keratinolytic activity in primary screening using skim milk agar medium among which, nine isolates showed keratinolytic activity using keratin agar medium. Isolate KA_{1a} gave the highest yield of keratinase and was identified as a strain of *Stenotrophomonas maltophilia* (NCBI Accession number MT478451), based on 16S rRNA sequencing. It delivered highest enzyme production (108 U/ml) in the medium with feathers (2.2%), NaCl (0.5%), K₂HPO₄(0.3%), KH₂PO₄(0.4%), pH7, with 10% inoculum of young cell mass, at 37°C for 24 h under shaking conditions. Optimum temperature for keratinase activity was 40°C and pH 7.0. Enzyme showed stability over different temperature and pH for up to 90 min. It showed potential applications as a detergent additive, animal feed, and organic fertilizer.

Keywords: Chicken feathers; keratinase; optimization; *Stenotrophomonas maltophilia*.

1.Introduction

The increased use of chicken, duck, and goose meat is having a negative impact on mother nature, as the feather waste generated from those chicks is not being properly degraded. Natural decay of feathers is slower, resulting in sulfurous substances that are harmful to the environment. Feathers generated in enormous volume in the form of by-product of poultry industries tends to be the store house of keratin (Adelere & Lateef, 2016). Keratin is the major component of feathers and is resistant to proteolytic enzymes namely papain, trypsin and pepsin.

Keratin persists as a structural component of vertebrate skin, hairs, feathers and other exoskeletons (Feughelman, 1985).

On the basis of their secondary structural conformations, keratin proteins are grouped as α helices (hair and wool) and β sheets (feathers) (Akhtar & Edwards, 1997).

Keratinase [E.C.3.4.21/24/99.11] proteases enzymes able to degrade the scleroprotein keratin. They are robust enzymes that work well at a number of

different temperatures and pH levels, with optimal activity near neutral to alkaline pH and temperature ranging between 40 and 70°C. These enzymes are also active over a variety of proteinic substrates such as keratin, fibrin, gelatin, and casein (Gupta & Ramnani, 2006; Lateef *et al.*, 2010). Keratinases are produced by bacteria, actinomycetes, and fungi which can hydrolyse a large number of keratin substrates (Tork *et al.*, 2010; Saibabu *et al.*, 2013).

Majority of feather-degrading bacterial isolates belong to gram-positive category of *Bacillus spp.* which includes *Bacillus subtilis*, *Bacillus cereus*, etc. (Adiguzal *et al.*, 2009; Kumar *et al.*, 2010). Keratinolytic bacteria that are gram negative, are the members of *Xanthomonas spp.*, *Stenotrophomonas spp.* etc. (De Toni *et al.*, 2002; Yamamura *et al.*, 2002). Keratin hydrolysate can be used in the medical fields, as animal feed, and addition as fertilizer, and as a detergent. The present study was aimed to isolate, identify, optimize and characterize the keratin degrading microorganisms from poultry farm soil and study their applications.

2. Materials and Methods

2.1 Sample collection

Soil samples (100g) were gathered from several feather dumping locations and chicken farms throughout Surat, India. (21.1790°N, 72.8020°E). The samples were collected and transported to the laboratory in a sterile plastic bag.

2.2 Enrichment of keratinase producing microorganisms

One gram of soil sample was mixed with 10ml distilled water. The mixture was shaken well and allowed to settle for 30 minutes. Five ml of soil supernatant inoculated in 95ml of whole feather basal broth medium: NaCl (0.5g/L), K₂HPO₄ (0.3g/L), KH₂PO₄ (0.4g/L), Feathers

(20g/L), pH 7.4 (Tork *et al.*, 2010). The flasks were incubated at 37°C at 120 rpm.

2.3 Isolation of keratinase producing microorganisms

Isolation of bacteria was performed by serial dilution using nutrient agar (NA) medium (Hi-media). The enriched suspension was transferred in 10 ml of sterilized distilled water. Aliquots, 0.1 ml of diluted sample was inoculated in nutrient agar plates from various dilution and spread uniformly. Plates were incubated at 37°C for 24-48 h. The bacterial isolates were sub-cultured on NA medium to obtain pure culture and maintained in NA slants at 4°C for further study.

2.4 Screening of keratinolytic bacteria

2.4.1 Primary screening

The isolates were firstly inoculated on skim milk agar (SMA) plates: peptone (5.0g/L), yeast extract (3.0g/L), dextrose (1.0 g/L), skim milk powder (10g/L), agar (15g/L), pH 7.2±0.2. The plates were incubated at 30°C for 48 h.

2.4.2 Secondary screening for keratinase activity

All positive primary screening isolates were subjected to secondary screening. Isolates were streaked on keratin agar (KA) plates: NaCl (0.5g/L), KH₂PO₄ (0.7g/L), K₂HPO₄ (1.4g/L), MgSO₄·7H₂O (0.1g/L), keratin powder (10g/L), agar (15g/L), pH 7.5. Plates were incubated at 37°C for 72h.

2.5 Identification and 16S rRNA sequencing

Identification was carried out based on colony characteristics, biochemical properties. Further confirmation of the strain was carried out using 16S rRNA sequencing analysis (Saffron life science Research Pvt. Ltd, Surat, India).

2.6 Production of keratinase

A 250 mL Erlenmeyer flask containing 90 mL Feather meal basal media was used to develop isolated colonies. Each flask was injected with ten millilitres of an overnight developed culture and cultured for seven days on a rotary shaker at 150 rpm. Feathers were noted to be deteriorating.

2.6.2 Assay for keratinase activity

The keratin digestion method was used to assess enzyme activity, with 1% keratin in 0.05M Tris-HCl buffer (pH 8.0) as the substrate. 1.75 mL substrate solution and 0.25 mL crude enzyme solution were used in the reaction mixtures. The reaction was stopped by adding 2.0 ml 0.4M trichloroacetic acid to the mixture and incubating it at 40 °C in a water bath for 10 minutes (TCA). The enzyme solution was incubated with 2.0 ml TCA without the addition of keratin as a control. After centrifuging the mixture for 30 minutes at 3825 rpm, the absorbance was measured at 280 nm using a spectrophotometer (Systronics) against a control. Gradiar *et al.*, (2005) defined one unit (U/ml) of enzyme activity as a 0.01 increase in absorbance at 280 nm per minute under test conditions calculated using equation [1].

$$U = 4 \times n \times A_{280} / (0.01 \times 10) \quad [1]$$

where n is the dilution factor; 4 is the final reaction volume (ml); 10 is the incubation time (min).

2.6.3 Protein estimation

Protein concentration was determined by using the Folin-Lowry Ciocalteu method. Bovine serum albumin (Hi-media) was used as a standard and the colour developed was read at 660 nm (Lowry *et al.*, 1951).

2.7 Media optimization

2.7.1 Inoculum preparation

Bacterial culture was inoculated in 10 ml nutrient broth, incubated in a shaker for 24 h at 37°C with 120 rpm and growth obtained was inoculated in 90 ml production media.

2.7.2 Substrate concentration

Different concentrations of feather viz. 0.9%, 1.3%, 1.6%, 1.9% and 2.2% was added in the production media. All the flasks were incubated at 37°C at 120 rpm. Samples were withdrawn every 24 h and were analysed for keratinase activity and protein content.

2.7.3 Incubation time

For determining the optimum time for keratinase production, the 90 ml of production medium was inoculated with 10 ml of inoculum, and flasks were incubated for different time duration i.e., 24 h, 48 h, 72 h, 96 h at 37°C at 120 rpm.

2.7.4 Effect of different nitrogen sources on keratinase production

The most suitable nitrogen source for keratinase production was determined by supplementing production media with 9 mg of various nitrogen sources namely yeast extract, urea, peptone, and tryptone.

2.7.5 Effect of concentration of nitrogen sources

Yeast extract was the most suitable nitrogen source for keratinase production supplemented with various concentrations i.e. 8-11 mg and without nitrogen source. All the flasks were inoculated with 10% inoculum and were incubated at 37°C at 120 rpm for 48 h.

2.7.6 Effect of pH on the production of keratinases

Adjusting the pH of the production medium at pH ranges from 5-9. The pH of the production medium was adjusted with 1M

NaOH and 1M HCL before inoculation. All the flasks were inoculated with 10% inoculum and were incubated at 37°C at 120 rpm for 48 h.

2.8 Characterization of crude enzyme

2.8.1 Determination of the optimum temperature

Effect of temperature on keratinase activity was checked by incubating the reaction mixture at temperature 30-60°C for 10 min and assayed for the enzyme activity.

2.8.2 Keratinases thermal stability

Thermal stability was examined by exposing the crude enzyme at different temperatures 30°C, 40°C, 50°C, 60°C for 90 min. Relative activity was calculated and % stability of the enzyme was determined.

2.8.3 Determination of the optimum pH

The optimum pH for enzyme activity was determined by incubating the reaction mixture at various pH ranges from 7-10 at water bath for 10 min at 40°C.

2.8.4 pH stability

Crude enzyme with 0.05M Tris-HCL buffer was incubated at various pH ranges from pH7-10 at room temperature for 90 min. The pH was adjusted with 1M NaOH and 1M HCL. Relative activity was calculated and % stability of enzyme was determined.

2.9 Application of keratinase

2.9.1 Keratinase enzymes as an additive in the detergents industry

Application of keratinase enzyme as a detergent additive was studied on white cotton cloth pieces (4×4 cm) stained with human blood and coffee. Seven mg/ml of Ariel detergent powder solutions were prepared and heated in a boiling water bath for 20 min to inactivate the inherent

enzymes. The following sets were prepared.

Flask with distilled water (100 ml) + stained cloth.

Flask with distilled water (100 ml) + stained cloth + 1 ml heat inactivated Ariel detergent (7mg/ml).

Flask with distilled water (100 ml) + stained cloth + 1ml heat inactivated Ariel detergent (7mg/ml) + 2 ml of enzyme solution.

The piece of cloth was rinsed in the above flasks for 30 min. After incubation, cloth pieces were taken out, rinsed with water, and dried. Visual examination of various pieces exhibited the effect of the enzyme in the removal of stains. Untreated cloth pieces stains were taken as control.

2.9.2 Keratinase enzymes as animal feeds

Degraded products of feathers were analysed for an amino acid profile by thin-layer chromatography (TLC) to analyse its potential as animal feed. In TLC, the developing solvent system was Butanol: Glacial acetic acid: water (12:3:5), and Ninhydrin solution was used as reagent (2gm of ninhydrin dissolved in 1000 ml acetone).

2.9.3 Keratinase enzymes as organic fertilizers

Two pots were taken and filled with soil and marked as A and Control. The seedling of *Vigna Radiate* (Mung bean) was transferred to pots. In a pot, A feather hydrolysate was added and in control, tap water was added. The pots were watered every day with the sample. Analysis of plants were done at a regular interval of the day.

2.10 Statistical analysis

All of the analytical experiments were done twice (two sets), and statistical relevance was determined by utilising R studio to do one-way ANOVA and Friedman Rank Sum Test.

3. Results

Five samples were collected from different poultry farms and feather dumping sites. A total of 16 isolates were obtained.

3.1 Screening of keratinolytic bacteria

A screening program was employed to obtain bacterial isolates capable of producing feather degrading extracellular keratinase enzyme using feathers as sole carbon and nitrogen source.

3.1.1 Primary screening

All 16 isolates were checked for their protease activity using skim milk agar plates. Only 10 bacterial isolates showed larger zones of clearance (Figure 1). This suggested that these isolates might possess keratinase activity as well.

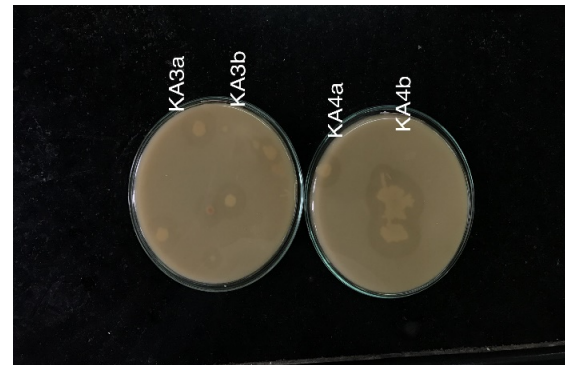
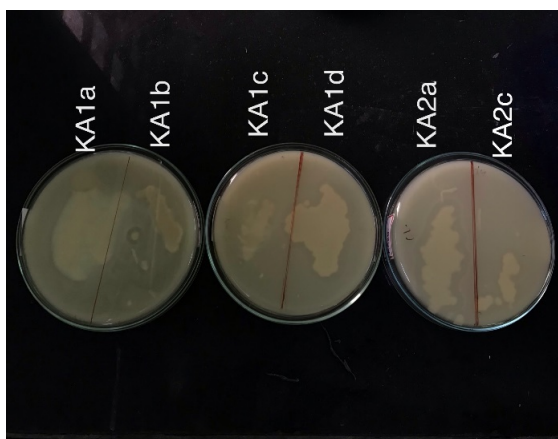


Fig. 1. Primary screening on SMA plates

3.1.2 Secondary screening

The above 10 isolates were grown on keratin agar plates for 24-48 h at 30°C. Among 10 bacterial isolates, 9 were found as keratinase producers based on their clear zone formation on keratin agar plates (Figure 2).



Fig. 2. Secondary screening on KA plates

The isolates that formed a definite clear zone on both the media were considered as keratinase-producing bacteria.

3.2 Production of keratinase enzyme

Bacterial isolates obtained from screening were cultivated in liquid medium containing sterile feather as substrate. After 24-48 h of incubation flasks showed turbidity (Figure 3).

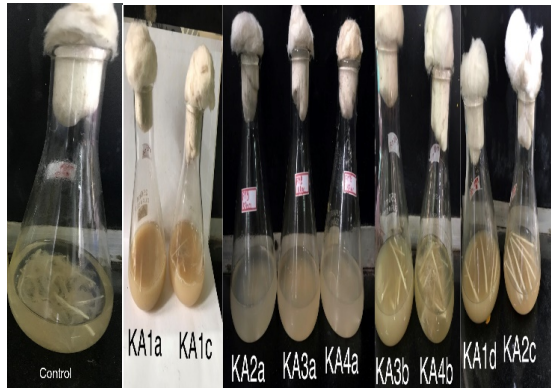


Fig. 3. Different isolates showing degradation of feathers

Degradation of feathers was visually observed.



Fig. 4. Successive feather degradation

All the analysis was performed in duplicates and the mean was considered as the final value. Standard deviation value ranging up to ± 3 was obtained throughout the studies.

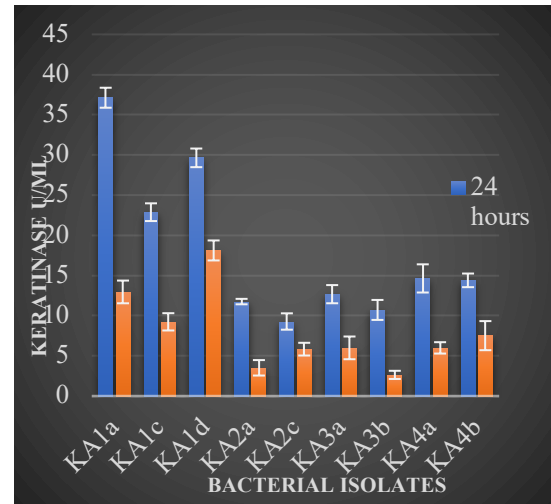


Fig. 5. Keratinase activity of different bacterial isolates. Values are means of 2 replicates \pm standard deviation.

Among all the isolated bacteria KA_{1a}, isolated from the Government of Gujarat poultry farm, Surat, India was found to be the highest keratinase producer with 37.12 U/ml keratinase activity after 24 h (Figure 5).

3.3 Identification and 16S rRNA sequencing

Identification of isolate KA_{1a} was carried out by studying the microscopic, morphological, physiological, and biochemical characteristics. The isolate was found to be Gram-negative rod-shaped motile bacteria. Biochemical analysis showed that isolate KA_{1a} was able to utilize citrate as well as demonstrated catalase activity.

Molecular identification performed by 16S rRNA sequencing and BLAST (with the help of saffron life science) showed close identity (97%) with *Stenotrophomonas maltophilia* strain NBRC 14161 (Figure 6). Hence, the isolate KA_{1a} was designated as *Stenotrophomonas maltophilia*.

Alignment with most coordinated sequence:

Stenotrophomonas maltophilia strain NBRC 14161 16S ribosomal RNA, partial sequence

Sequence ID: [NR_113648.1](#) Length: 1470 Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
555bits(300)	7e-158	320/330(97%)	0/330(0%)	Plus/ Plus

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Query 1  GTTGGCGGGGTAAGGCCACCAAGGGGACAAATCCGTAGCTGGTCTGAGAGGATGATCAC
          |||
Sbjct 224 GTTGGCGGGGTAAGGCCACCAAGGGGACGATCCGTAGCTGGTCTGAGAGGATGATCAG

Query 61  CCACACTGGAAGTCCAGACCGTCCACACTCCTACGGGAGGCAGTGGGGAATATTGG
          |||
Sbjct 284 CCACACTGGAAGTCCAGACCGTCCAGACTCCTACGGGAGGCAGTGGGGAATATTGG

Query 121 ACAATGGGGCAAGCCTGATCCACCCATACCGCGTGGTGAAAAAAGCCTTCGGGTGTA
          |||
Sbjct 344 ACAATGGGGCAAGCCTGATCCAGCCATACCGCGTGGTGAAGAAGGCCTTCGGGTGTA

Query 181 AAGCCCTTTTGTGGGAAAGAAATCCAGCTGGTAAATACCCGGTGGGATGACGGTACCC
          |||
Sbjct 404 AAGCCCTTTTGTGGGAAAGAAATCCAGCTGGTAAATACCCGGTGGGATGACGGTACCC

Query 241 AAAGAATAAGCACCAGGCTAACTTCTTCCAGCACCAGCGGTAATACAAAAGGTGCAAGCG
          |||
Sbjct 464 AAAGAATAAGCACCAGGCTAACTTCTTCCAGCACCAGCGGTAATACAAAAGGTGCAAGCG

Query 301 TTACTCGGAATTACTGGGCGTAAAGCGTGC 330
          |||
Sbjct 524 TTACTCGGAATTACTGGGCGTAAAGCGTGC 553
    
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Fig. 6. 16S rRNA partial sequence

3.4 Optimization of process parameter

Optimization of keratinase production from the selected bacterial isolate was done by evaluating the effects of the following parameters.

3.4.1 Substrate concentration

Five different substrate concentrations were considered viz. 0.9,1.3,1.6,1.9 & 2.2%. The result (Figure 7(a)) depicted that an increase in keratinase yield relative to increased substrate concentration. The highest amount of keratinase was obtained with the substrate concentration of 2.2% in the production medium with 109.65 U/ml keratinase activity.

Substrate concentration is significant and was confirmed statistically by ANOVA which suggested the Degree of freedom 1, Mean sum of squares 508.9 with P value 0.002(<0.05).

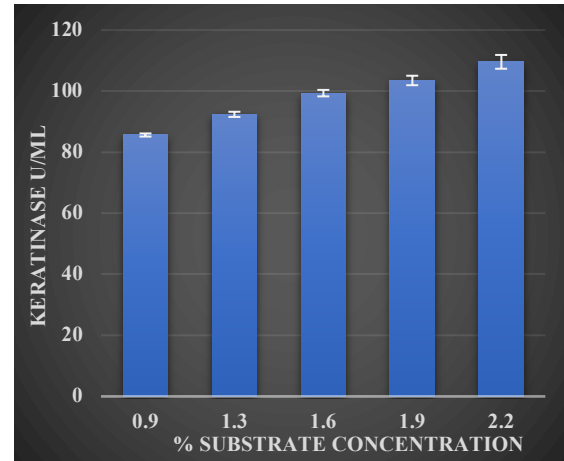


Fig. 7(a). Effect of Substrate concentration on Keratinase production. Values are means of 2 replicates ± standard deviation.

3.4.2 Incubation time

The bacterial cultures were incubated at different time ranges from 24h -96 h. It was observed that (Figure 8) keratinase production increased within 24h. Keratinase activity 102.76 U/ml was detected at 24 h, after which it decreased.

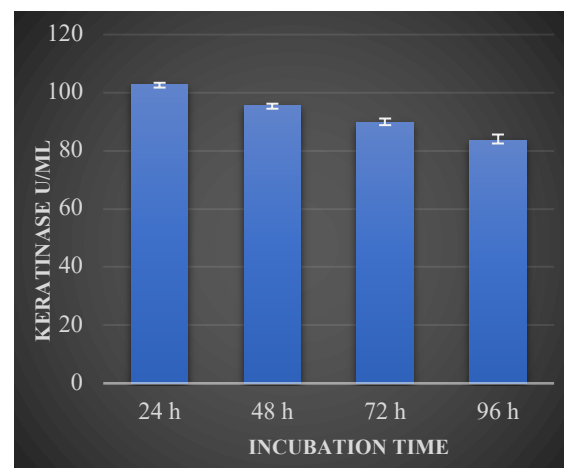


Fig. 8. Incubation time for Keratinase production. Values are means of 2 replicates ± standard deviation.

3.4.3 Effect of different nitrogen sources on the production of keratinases

Among various nitrogen sources viz. yeast extract, urea, peptone, and tryptone, yeast extract was the best nitrogen source for keratinase production 82.4 U/ml (Figure 9).

One-way ANOVA for statistical analysis of the effect of incubation time on keratinase production was performed and it showed that it was significant providing a p-value of 0.000538 (<0.05) with a degree of freedom 3 and mean sum of square 134.17.

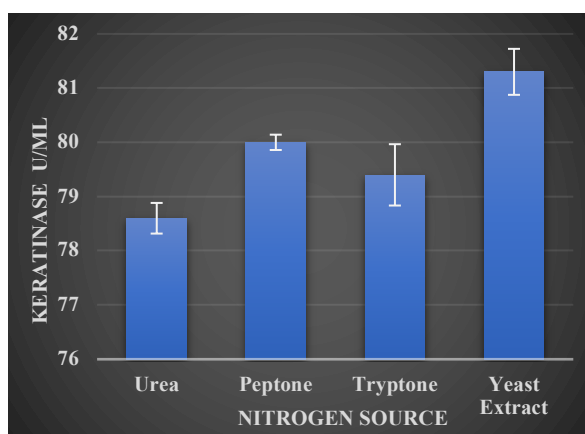


Fig. 9. Effect of nitrogen sources on Keratinase production. Values are means of 2 replicates \pm standard deviation.

3.4.4 Effect of concentration of nitrogen sources

The results (Figure 10) depicted an increase in keratinase yield without a nitrogen source. Feathers themselves act as nitrogen sources. The isolate accumulated the highest keratinase in the production medium having without nitrogen source with 108 U/ml.

The effect of nitrogen concentration was significant which was confirmed by applying ANOVA which stated the mean sum of square 113.71 and degree of freedom 4 producing p-value of 0.00046.

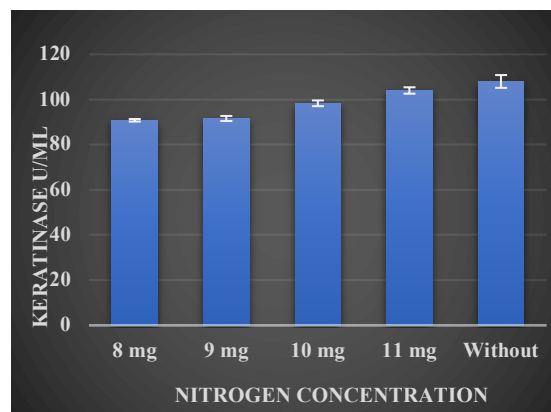


Fig. 10. Effect of nitrogen source concentration on Keratinase production. Values are means of 2 replicates \pm standard deviation.

3.4.5 Effect of pH on the production of keratinases

The influence of initial medium pH on keratinase production was analysed. At pH 7, the highest amount of keratinase was produced (92.84 U/ml). (Figure 11). Results of ANOVA suggested statistical relevance by generating P-value of 0.00029 (<0.05), Degree of freedom 3 and Mean sum of squares 189.11.

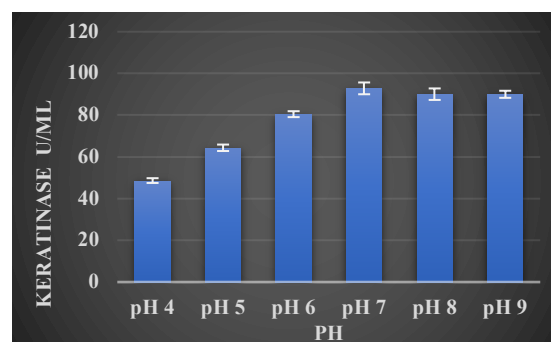


Fig. 11. Effect of pH on Keratinase production. Values are means of 2 replicates \pm standard deviation.

3.5 Characterization of enzyme

3.5.1 Determination of the optimum temperature

Keratinase activity was observed at 30-60°C. The optimum temperature for keratinase activity was found to be 40°C (Figure 12).

ANOVA for the effect of temperature on keratinase activity was suggested to be nonsignificant providing Degree of Freedom 1 and Mean sum of squares 56.69 with a p-value of 0.4. Hence it can be stated that enzyme showed activity over a wide range of temperatures.

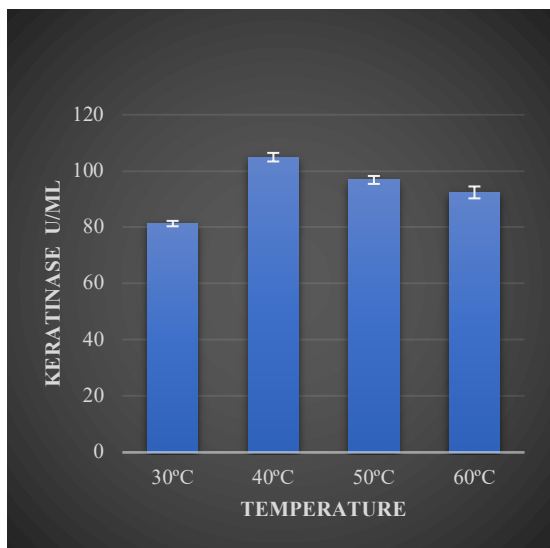


Fig. 12. Effect of temperature on keratinase activity. Values are means of 2 replicates \pm standard deviation.

3.5.2 Keratinases thermal stability

The activity before incubation was taken as 100%. The enzyme was very stable up to 50°C followed by a loss of activity above 60°C. The enzyme retained more than 90% and 80% of its activity at 40°C and 50°C (Figure 13).

Statistical analysis performed by Friedman Rank Sum Test gave the Friedman chi-squared value 12 and p-value 0.007 which was less than 0.05. Hence it was significant.

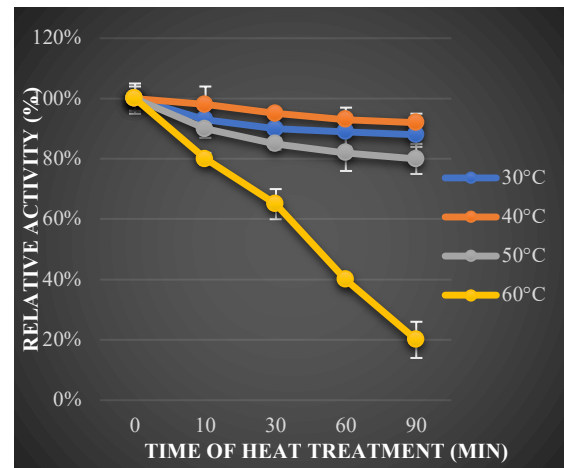


Fig. 13. Temperature stability of Keratinase. Values are means of 2 replicates \pm standard deviation.

3.5.3 Determination of the optimum pH

The enzyme was most active in the pH range of 7.0 to 9.0, with 7.0 being the optimal pH. (Figure 14). The effect of pH on keratinase activity was found to be statistically insignificant through ANOVA by generating a p-value more than 0.05. This can be due to the significant activity of keratinase over more than one pH.

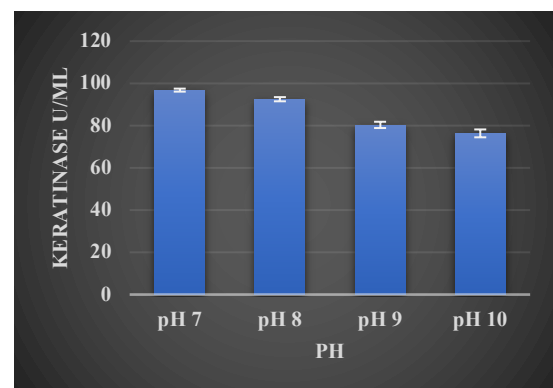


Fig. 14. Effect of pH on keratinase activity. Values are means of 2 replicates \pm standard deviation.

3.5.4 pH stability

When exposed at pH 7, the enzyme retained 91% of its activity and maintained more than 77% of its original activity in the pH range 8-10 (Figure 15). Friedman Rank

Sum Test was performed for analyzing the statistical significance of keratinase stability over various pH and the results generated a p-value less than 0.05 suggesting the stability was significant.

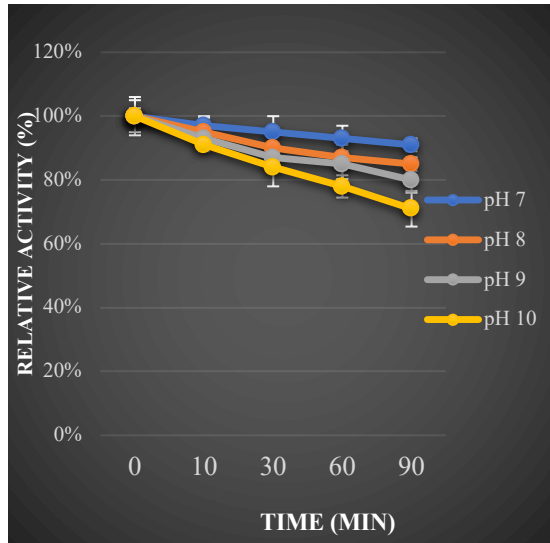


Fig. 15. pH stability of Keratinase. Values are means of 2 replicates \pm standard deviation.

3.6 Application of keratinase

3.6.1 Keratinase enzymes as an additive in the detergents industry

Visual observations were made after subjecting the stained cloth pieces to various control and test solutions (Figure 16a and 16b). Results indicate that keratinase was able to de-stain both blood and coffee stains on the cloth as shown in Figure 16a[C] and Figure 16b[C] compared to wash performance of the detergent Figure 16a[B] and Figure 16b[B].

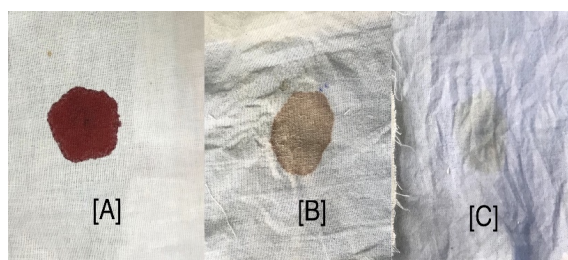


Fig. 16(a) [A] Control: bloodstain rinsed by distilled water [B] Blood +Ariel: bloodstain rinsed in Ariel detergent solution [C] Blood + Ariel + Enzyme: bloodstain rinsed by detergent followed by enzyme

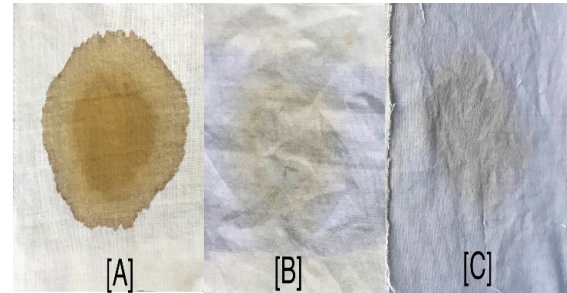


Fig. 16(b) [A] Control: coffee stain rinsed by distilled water. [B] Coffee +Ariel: coffee stain rinsed in Ariel detergent solution [C] Coffee + Ariel + Enzyme: coffee stain rinsed by detergent followed by enzyme

3.6.2 Keratinase enzymes as animal feeds

TLC analysis of the products of the feather degradation showed the presence of amino acids, histidine, lysine, and tryptophan (Figure 17). This was determined based on RF values.

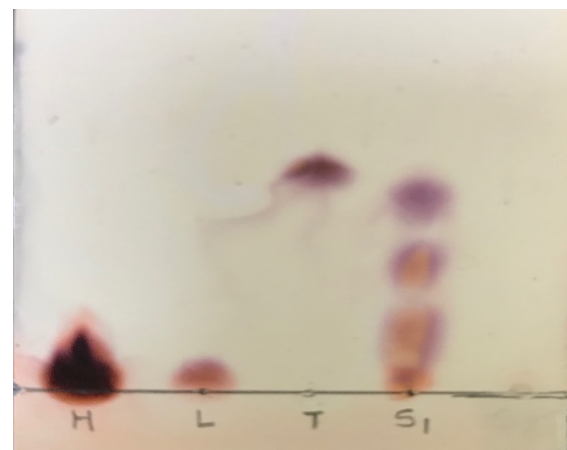


Fig. 17(a) TLC of amino acids released from degraded feathers

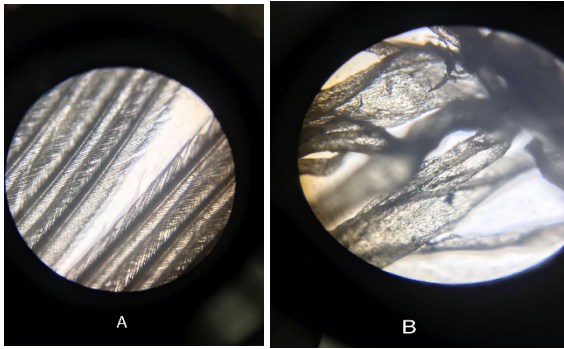


Fig. 17(b) Microscopic observation [A] Feather [B] Degraded feather

3.7.3 Keratinase enzymes as organic fertilizers

Effect of feather hydrolysate on the growth of *Vigna radiate* plant as shown in figure 18a and 18b. Results indicate that Pot A plant showed more height than control.



Fig. 18(a) [A] Growth of plant treated with Feather hydrolysate [Control] Growth of plant treated with tap water

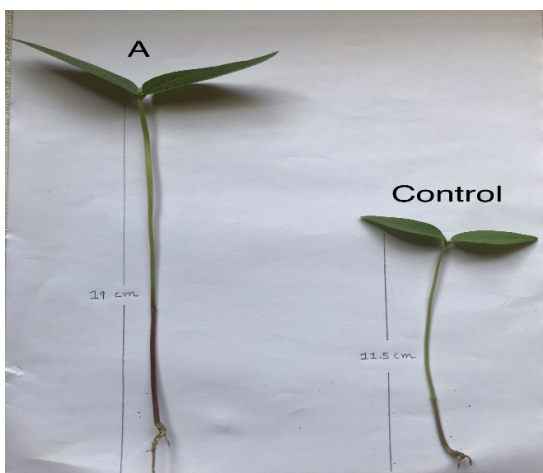


Fig. 18(b) [A] Height of plant treated with feather hydrolysate [Control] Height of plant treated with tap water.

4. Discussion

In the current investigation, bacteria were isolated from soil samples and evaluated for keratinase production. Selected isolate KA_{1a} was gram-negative rod-shaped bacteria and was confirmed as a strain of *Stenotrophomonas maltophilia*.

Stenotrophomonas maltophilia is a dominant keratinase-producing taxa. Strains of this species have been frequently isolated from poultry and feather waste dumping site which contains a rich source of the protein keratin. It is capable of the effective degradation of bird feathers and depilation of animal skins through the action of enzyme keratinase (Yamamura *et al.*, 2002, Cao *et al.*, 2009, Jeong *et al.*, 2010).

The success of the microbial enzyme production process highly depends on certain Physico-chemical factors such as incubation time, substrate concentration, nitrogen source, pH of the medium, etc. All these factors are responsible for the optimum growth of microorganism which can directly affect enzyme production. Hence their optimum value must be determined to achieve the maximum possible enzyme yields.

Substrate concentration can be considered as one of the key factors for microbial keratinase production. It plays a dual role as carbon source as well as an inducer for enzyme production. Here feather meal because of its keratin-rich component, act as an inducer for keratinase production simultaneously along with providing carbon for growth and metabolism. Decreased substrate concentrations fail to deliver nutrients needed for appropriate growth, resulting in famine, whereas high

feather concentrations have been shown to produce substrate inhibition or repression of keratinase production, resulting in a lower proportion of feather breakdown. Shah & Vaidya, (2017) showed maximum keratinase production with 1% and 1.5% concentration.

Microorganisms require a suitable nitrogen source as it is an inseparable part of cellular components and biomolecules. It has been reported previously, *B. pumilus* FH9, *Bacillus megaterium* F7, and *Stenotrophomonas maltophilia* R13 exhibited maximum keratinase production in presence of yeast extract (El-Refai *et al.*, 2005; Park & son 2009; Jeong *et al.*, 2010). In this study feathers, are a rich source of protein keratin which has a dual advantage of providing nitrogen along with the carbon for the growth of bacteria. This makes the process economical as no additional nitrogen source is required to be added to the media. Keratin in the waste chicken feathers is a cheaper alternative to peptone and also a nitrogen source for microbial growth (Akpoy *et al.*, 2018).

Changes in pH affect bacterial growth simultaneously with enzyme production. Moreover, it can hinder the availability of substrate for enzymes by altering their properties. Jeong *et al.*, (2010), reported the highest keratinase production by *Stenotrophomonas* at pH 7.0. pH 7.0 being neutral is the most favourable pH for bacterial growth and hence, would have contributed to higher keratinase production.

Characterization of the enzyme leads to a determination of optimum conditions for enzyme action. Temperature and pH are crucial for enzyme activity. At optimum temperature and pH, an enzyme shows maximum catalytic activity. Temperature rise beyond a certain point can cause a rapid decline in the enzyme activity due to enzyme denaturation. Thermal stability deciphers enzyme's ability to maintain its structure as well as activity at high

temperatures. Thermostable enzymes have commercial value as they can lower the cooling cost of the process. According to Meng *et al.*, 2013, keratinase is most active at 40°C. Any change in pH has an impact on protein structure, and a drop in enzyme activity beyond its optimal pH could indicate deactivation or instability of the enzyme. Cai *et al.*, (2008) reported that *Microbacterium* kr10 keratinase showed maximum activity at pH 7.

Keratinase can provide a solution to waste disposal generated from poultry industries. Additionally, the protein-digesting enzyme has great potential as a detergent additive to clear the proteinic stain. Also, it can generate a mixture of amino acids, which can provide better nutrients in animal feed. The use of feather meals as animal feed has been reported (Riffel *et al.* 2003).

As well, it can increase the growth of plants if used as an organic fertilizer. The addition of feather hydrolysate to soil has been shown to increase urease and protease enzyme activity. Proteases and ureases in the soil play a significant role in nitrogen mineralization. Feather being rich in protein their microbial degradation in soil would release peptides and amino acids which may stimulate soil microbial activity, thereby facilitating the assimilation of nutrients by plants and better growth.

5. Conclusion

Among different microbial isolates screened for keratinase production using feathers, a newly isolated strain of *Stenotrophomonas maltophilia* with accession no. MT478451 was found to be the most potent producer. This organism was able to produce keratinase in feather basal medium supplemented with Feathers (2.2%), MgSO₄.7H₂O (1%), NaCl (0.5%), NH₄Cl (0.5%), K₂HPO₄ (0.3%), pH 7, inoculated with 1% v/v pre-grown cell mass and incubated at 37°C under shaking conditions. Optimization studies increased

keratinase yield by 2.9 fold giving 108 U/ml. Keratinase produced showed maximum activity at pH 7.0 and 40°C and demonstrated high pH and temperature stability up to 90 min. Keratinase showed promising effect with a commercial washing a detergent, animal feed, and organic fertilizer. Hence, *Stenotrophomonas maltophilia* (KA_{1a}) has the potential for producing a significant amount of keratinase which possesses commercially desirable properties.

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