



---

## PRODUCTION OF KERATINASE FROM FEATHER DEGRADING STENOTROPHOMONAS MALTOPHYLA AB20

A. R. Birari<sup>1</sup>, K. P. Narkhede<sup>1\*</sup> and N. S. Gaikwad<sup>2</sup>

<sup>1</sup>Department of Microbiology, M.J. College, Jalgaon-425002, India.

<sup>2</sup>Department of Microbiology, Toshniwal A.C.S.College, Sengaoon Dist. Hingoli(MS), India

Corresponding author: K.P. Narkhede and A.R. Birari.

Email: kpn.mjc@gmail.com ; anilbirari.021@gmail.com.

---

### ABSTRACT :

Keratinolytic bacteria *Stenotrophomonas maltophilia* AB20 have feather degrading ability which is isolated from feather waste soil, thus bacterial strains were identified by physical, biochemical characteristics, and 16S rRNA sequences. Keratinase production maximized through medium optimization of the selected keratinase producing isolate *Stenotrophomonas maltophilia* AB 20, and production checked through submerged fermentation with various single parametric factors. Maximum keratinase production was obtained with incubation period at 6<sup>th</sup> day 75 U/ml of final production medium, with each effective factor like Temperature 30°C (72 U/ml), pH 7 (68 U/ml), Agitation rate 150 rpm (63.2U), Carbon source Maltose (65U/ml), Organic nitrogen source Yeast extract (63.7U/ml), Inorganic nitrogen source Sodium nitrate (60.8U/ml) and Activators metal ions MgCl<sub>2</sub> (58U/ml) that were tested with specific keratinase activity. Keratinases which are produced by these keratinolytic organisms could be used to degrade keratinous waste and further the digested products could be an excellent material for producing animal feed and fertilizers.

**KEYWORDS :** Feather waste, *Stenotrophomonas maltophilia*, Keratinase production.

### INTRODUCTION

Poultry processing plant produced million tones of feather waste as by product, and it creates environmental pollution. Keratin is an insoluble macromolecular protein which is resistance to extra cellular proteases for biodegradation. Keratins secondary structural protein packed tightly either in  $\alpha$ -helix or in  $\beta$ -sheet structures linked by disulphide bonds through responsible for the stability of keratin. Keratins are two types hard keratins (feather, hair, hoof and nail) and soft keratins (skin and callus) (Gradisar, *et al.*, 2005). The degradation of keratinous material is important medically and agriculturally, a current use of feathers is conversion to feather meal, a digestible dietary protein for animal feed, using physical and chemical treatments. These methods can destroy certain amino acids and decrease biological value of protein quality and digestibility (Brandelli *et al* 2010). Secretion of keratinolytic enzymes is associated with number of soil microorganisms or which recalcitrant keratin protein. It is degraded a number of feather degrading species belong to bacteria, actinomycetes and fungi have been used for the production of keratinase enzyme in submerged as well as in solid state fermentation. Most of Keratinase enzymes from microbial sources are extracellular and inducible by keratin waste through submerged fermentation (Gupta and Ramnani, 2006). However, much current research is centered on the potential use of keratinase of bacterial origin for the industrial treatment of keratin-containing compounds, *Pseudomonas* sp. (Linang *et al* 2011) keratinolytic enzyme by a newly isolated *Stenotrophomonas maltophilia* R13 that produces plant growth-promoting activity (Jeong *et al* 2010). These enzyme finds important application in leather industry, a biological

approach could be advantages over the thermal and chemical methods since it is a friendly environment and energy conservation process resulting in its feather products could have a potentially valuable source of protein as animal. The present study describes the production of Keratinase for single factor parametric approaches produced by *Stenotrophomonas* sp. AB 20 isolated from poultry waste.

## MATERIALS AND METHODS

Isolation and identification of Feather-degrading bacteria were employing enrichment culture technique from the soil. Primary and secondary screening approach, Individual colonies with clear zones resulting from proteolysis were picked and grown basal feather medium containing 1% chicken feather as the sole carbon and nitrogen source. During the cultivation at 30°C and at 120 rpm, keratinolytic activity and feather degradation rate were checked at 24 hr time intervals. The isolate exhibiting the highest activity was chosen for further investigation. The basal feather medium used in this study comprised of (g/L) NaCl 0.5, K<sub>2</sub>HPO<sub>4</sub> 1, KH<sub>2</sub>PO<sub>4</sub> 0.6, MgCl<sub>2</sub>.6H<sub>2</sub>O 0.1, Feather 10, pH 7.5. It was incubated at 30°C for 5 days at 120 rpm (Brandelli A, Riffel 2005; Agrahari and Wadhwa 2010)

Morphological and cultural properties of the strain AB 20 were studied according to methods for general and molecular bacteriology. The sequence of 16S rRNA gene was determined after genomic DNA extraction and polymerase chain reaction, amplification based on a previous work. Databases in GenBank were searched for sequences similar to the 16S rRNA gene sequence. The nucleotide sequence determined in this work has been deposited in GenBank database with accession number KF201645 (Birari et al 2013)

Medium improvement for keratinolytic enzyme production to investigate the effect of culture conditions for the keratinolytic enzyme production cultured over a range of Initial pH range 5.0–10 (Fig.1), Temperature range 20–55°C (Fig.2), In order to various carbon sources glucose, maltose, sucrose, fructose, starch, xylose, mannose, arabinose, lactose (Fig.3) then nitrogen sources peptone, yeast extract, meat extract, soya peptone, tryptone, urea (Fig.4) In organic nitrogen source ammonium nitrate, ammonium chloride, ammonium sulphate, sodium nitrate, potassium nitrate (Fig.5), metal ions CaCl<sub>2</sub>, BaCl<sub>2</sub>, MgCl<sub>2</sub>, FeCl<sub>3</sub>, CoCl<sub>2</sub>, MgSO<sub>4</sub>, MnSO<sub>4</sub>, ZnSO<sub>4</sub>, CuSO<sub>4</sub>, AlSO<sub>4</sub> (Fig.6) All these medium were supplemented individually to the basal feather medium containing 1% chicken feather with respective final concentration of 0.1% for above organic and inorganic source respectively and effect of different feather concentrations (0.1–2%) on the keratinolytic enzyme production was also investigated (Fig.7). And finally Enzyme production period also investigated (Fig.8).

For the production of keratinolytic enzyme, 50 ml of the medium was dispensed into each of 250-ml Erlenmeyer flasks followed by inoculation with 1ml of the strain AB 20 culture ( $1.4 \times 10^4$  cells/ml) grown in nutrient broth at 30 °C for 8 h. Cultivations were carried out at 30°C and 120 rpm for 3 day in a rotary shaker. Assay of keratinolytic activity and soluble protein was assayed spectrophotometrically by using Azokeratin as substrate the culture supernatants obtained by centrifugation at 10000 rpm for 15 min. one unit of Enzyme activity is defined as increase in absorbance at 440 nm with 0.01 U/ml the Keratinase assayed was prepared by the method as described previously (Brandelle et al 2005). Soluble protein concentration was determined by Lowery method (Lowery 1951) with bovine serum albumin as the standard. All experiments were repeated three times.

## RESULTS

The keratinolytic activity of the strain *Stenotrophomonas maltophilia* AB 20 was assayed in the basal feather medium containing 1% chicken feather as sole carbon and nitrogen source. The level of keratinolytic activity in the medium showed maximum after 48 h of incubation.

The influence of initial pH on the of *Stenotrophomonas maltophilia* AB 20 keratinolytic enzyme production was investigated in optimum at pH 7.0 (68 U/ml) Fig.1 The influence of temperature on the keratinolytic enzyme production observed optimum level of production was achieved at 30 °C. (72 U/ml) Fig.2. Optimum enzyme activity also observed at 150 rpm Agitation rate (63.2 U/ml). In order to study the influence of co-carbon sources on the keratinolytic enzyme production, various co-carbon sources were

added to the basal feather medium Maltose promoted the greatest degree of keratinolytic enzyme production (65 U/ml) Fig. 4. To investigate the influence of co-nitrogen sources on the keratinolytic enzyme production the highest keratinolytic enzyme production was obtained with Yeast Extract (63.7 U/ml) Fig. 5 and In organic nitrogen source Sodium nitrate (60.8U/ml) As shown in Fig. 6 The influence of different Metal ions on the keratinolytic enzyme production was investigated. Maximal keratinolytic enzyme production was found in  $MgCl_2$  (58U/ml) Fig. 7. And finally enzyme production of optimized medium is 75 U/ml at 4<sup>th</sup> day Fig. 8 as compared to an optimized medium 52.82U/ml increase 1.41 fold keratinase production

## DISCUSSION

Similar report of keratinase production strain *Pseudomonas* sp. MS 21 was reported to produce optimum keratinase production 43 U/ml Optimum temperature is 37 °C and optimum pH 8, Mannitol and  $CaCl_2$ ,  $ZnCl_2$  decrease enzyme activity (Tork et al 2010). Similarly approach *Stenotrophomonas maltophilia* R13 use feather as sole carbon and nitrogen source for minimal medium and 0.1% Glucose, 0.12% polypeptone increase enzyme production, optimum pH and Temperature is 7 and 30°C respectively (Jeong et al 2010) Maximum Keratinase production recorded at 40°C and initial optimum pH is 7.5-8, metal activator  $Zn^{2+}$  activate most of keratinase activity and inhibited by heavy metal ion like  $Pb^{2+}$ ,  $Cd^{2+}$ ,  $Hg^{2+}$  (Cao et al 2009). Similarly  $Ba^{2+}$ ,  $Ca^{2+}$  activate keratinase production (Lin et al 2009) With regard to optimum temperature *Pseudomonas aeruginosa* at 40°C (Raju et al 2013) *Bacillus thuringiensis* TS2 with 50°C (Sivakumar et al 2012). *Bacillus weihenstephanensis* PKD 5 required 120 rpm (Sahoo et al 2012) the optimum amount of inoculums was found to be *Pseudomonas aeruginosa* produced keratinase with 5% (Raju et al 2013). The carbon source, glucose was found to be good co substrate maximum keratinase activity. Among nitrogen sources maximum keratinase was produced *Bacillus pumilus* A1 yeast extract 47.68 U/ml (Zouari et al 2010).

## CONCLUSION

The isolate *Stenotrophomonas maltophilia* AB 20 produced a significant amount of Keratinase under submerged fermentation. Organism grows on simple media with feathers as its sole carbon, nitrogen, and energy source. The above report stated the evidence for the production of keratinase with substrate interactions of bacterial strains with maximum keratinase production 75 U/ml at 4<sup>th</sup> day with 1.41 fold keratinase production simple and effective manner. At the same time, it transforms industrial waste (chicken feather) into nutritional feed additives and protects the environment. Thus, utilizing poultry feathers as a fermentation substrate in conjunction with keratin-degrading microorganism or enzymatic biodegradation may be a better alternative to improve nutritional value of poultry feathers and reduce environmental waste.

## Figures

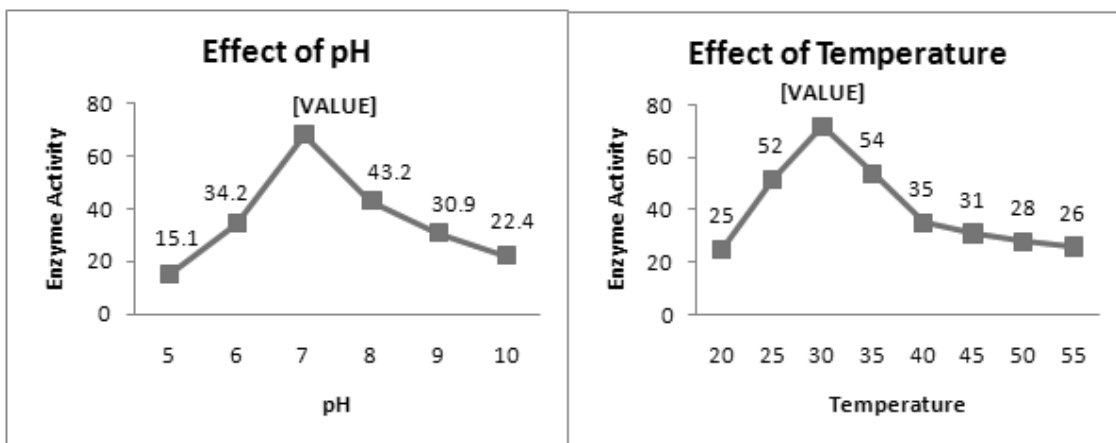


Fig. 1

Fig. 2

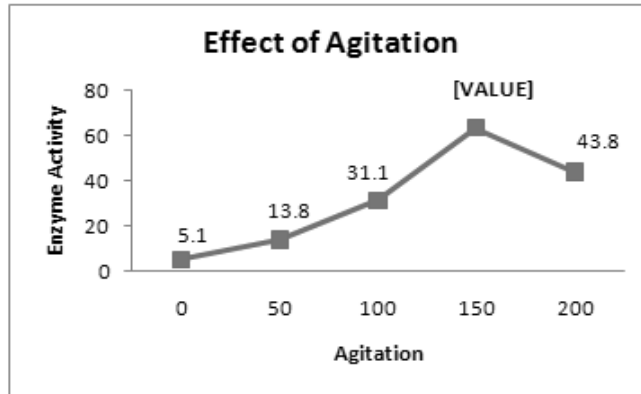


Fig. 3

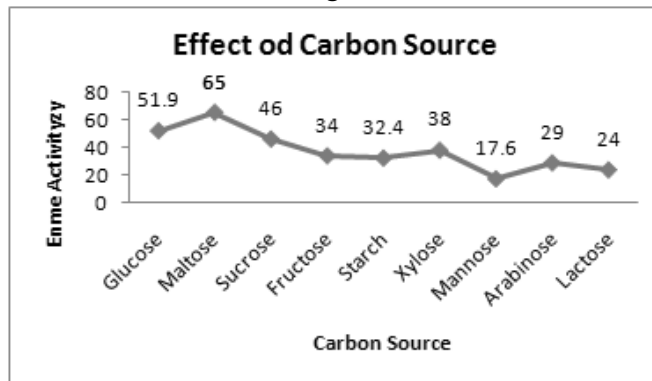


Fig. 4

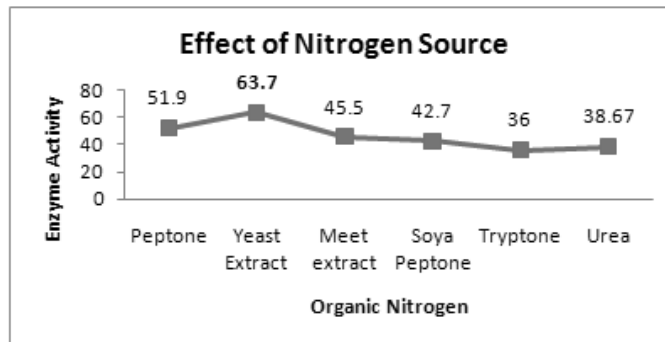


Fig. 5

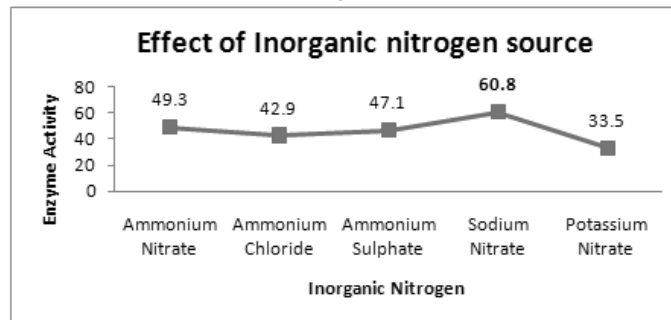


Fig. 6

Fig. 7

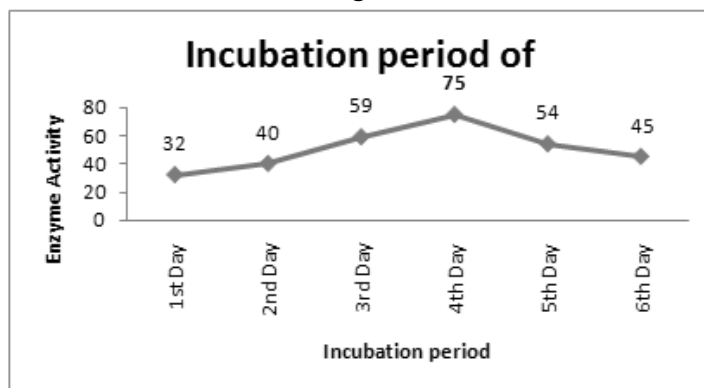
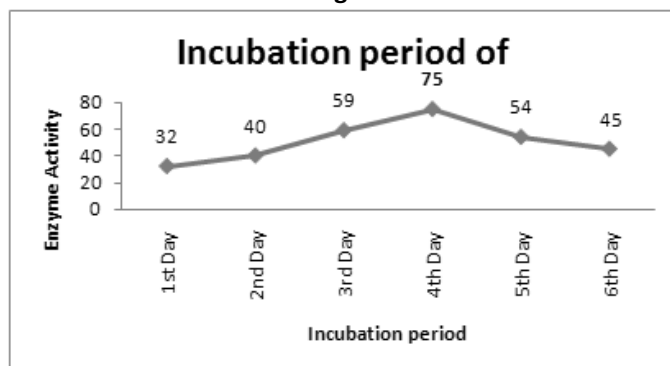


Fig. 8



## REFERENCES

1. **Agrahari S, Wadhwa N** (2010) Degradation of Chicken Feather A Poultry Waste Product By Keratinolytic Bacteria Isolated From Dumping Site At Ghazipur Poultry Processing Plant. *International Journal of Poultry Science* 9 (5): 482-489.
2. **Birari AR, Narkhede KP, Salunke RS** (2013) Feather Keratin Degradation by *Stenotrophomonas* sp. AB20 Screened from Poultry Waste. *Journal of Pure And Applied Microbiology*, December 2013. Vol. 7(4), p. 2973- 2979
3. **Brandelli A, Riffel A** (2005) Production of an extracellular keratinase from *Chryseobacterium* sp. growing on raw feathers. *Electronic Journal of Biotechnology* 8 (1): 35-42.
4. **Brandelli A, Daroit DJ, Riffel A** (2010) Mini-Review: Biochemical features of microbial keratinases and their production and applications. *ApplMicrobiolBiotechnol* 85: 1735–1750.
5. **Cao ZJ, Zhang Q, Wei DK, Chen L, Wang J, Zhang XQ, Zhou MH** (2009) Characterization of a novel *Stenotrophomonas* Isolate With High Keratinase Activity And Purification Of The Enzyme. *J IndMicrobiolBiotechnol* 36: 181–188.
6. **Gradisar H, Friedrich J, Krizaj I, Jerala R** (2005) Similarities and Specificities of Fungal Keratinolytic Proteases: Comparison of Keratinase of *Paecilomycesmarquandii* and *Doratomycesmicrosporus* to Some Known Proteases. *Applied Environmental Microbiology* 71 (7): 3420–3426.
7. **Gupta R, Ramnani P** (2006) Mini-Review:Microbial keratinases and their prospective applications: an overview. *ApplMicrobiolBiotechnol* 70: 21–33.
8. **Jeong EJ, Rhee MS, Kim GP, Lim KH, Yi DH, Bang BH** (2010) Purification and Characterization of a Keratinase from a Feather-degrading Bacterium, *Bacillus* sp. SH-517. *J. Korean Soc. Appl. Biol. Chem.* 53 (1): 43-49.

9. **Liang JD, Han YF, Zhang JW, Du W, Liang ZQ, Li ZZ** (2011) Optimal culture conditions for keratinase production by a novel thermophilic *Myceliophthora thermophila* strain GZUIFR-H49-1. *Journal of Applied Microbiology* 110: 871–880.
10. **Lin HH, Yin LJ, Jiang ST** (2009) Functional Expression and Characterization of Keratinase from *Pseudomonas aeruginosa* in *Pichia pastoris*. *J. Agric. Food Chem.* 57 (12): 5321–5325.
11. **Lowry O H, Nira J, Rosebrough A, Lewis F, Rose JR** (1951) Protein Measurement With The Folin Phenol Reagent. *J. Biol. Chem* 193: 267-275.
12. **Raju EVN, Divakar G.** (2013) Production Of Keratinase By Using *Pseudomonas aeruginosa* Isolated From Poultry Waste. *IJPCBS* 3: (1) 79-86.
13. **Sahoo DK, Das A, Thatoi H, Mondal KC, Mohapatra PKD** (2012) Keratinase Production and Biodegradation of Whole Chicken Feather Keratin by a Newly Isolated Bacterium Under Submerged Fermentation. *ApplBiochemBiotechnol* 167:1040–1051.
14. **Sangali<sup>a</sup> S, Brandelli A** (2000) Feather keratin hydrolysis by a *Vibrio* sp. strain kr2. *Journal of Applied Microbiology* 89: 735-743.
15. **Sivakumar T, Shankar T, Vijayabaskar P, Ramasubramanian V** (2012) Plant growth promoting activity of nickel tolerant *Bacillus cereus* TS1. *Journal of Agricultural Technology* 8 (6): 2101-2113.
16. **Tork S, Aly MM, and Nawar L** (2010) Biochemical and Molecular Characterization of a New Local Keratinase Producing *Pseudomonas* sp., MS21. *Asian Journal of Biotechnology* 2 (1): 1-13.
17. **Zouari NF, Hmidet N, Haddar A, Kanoun S, Nasri M** (2010) A Novel Serine Metallokeratinase from a Newly Isolated *Bacillus pumilus* A1 Grown on Chicken Feather Meal: Biochemical and Molecular Characterization. *ApplBiochemBiotechnol* 162: 329–344.