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Keratin degradation by bacterial strain isolated from poultry farm soil

Manoj Kumar, Rajesh Kumar and Deepak Kumar Malik*
Department of Biotechnology Engineering, UIET, Kurukshetra University, Kurukshetra-136119, Haryana, India.

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ABSTRACT

Objective: The aim of the current study was to isolate keratinolytic bacteria from the soil samples collected from poultry farm. **Background:** The awareness of importance of microbial degradation of keratin substrate into usable products many efforts are being conducted to isolate keratinolytic microorganisms. **Methods:** The isolation was performed by using enrichment technique. Total twenty four bacterial strains were isolated from the collected soil samples. All isolates were screened for proteolytic activity by skim milk agar plate method, among twenty four bacterial isolates. **Results:** Out of 24 only 6 isolate were showing Proteolytic activity as indicated zone of clearance on skim milk agar plate. Out of these 6 Proteolytic strains only 2 strains were showing keratin degradation ability by using chicken feather as a sole carbon source in feather meal broth. Finally out of two isolates MKR9 bacterial strain was selected for further study. **Conclusion:** Isolated bacterial strain producing keratinases is prominence in degradation of poultry waste feather. These keratinase producing microbial strains have an important application in removal of poultry waste and recycled into valuable by-product.

KEYWORDS: Keratinolytic bacteria, Proteolytic activity, Feather, Poultry waste.

INTRODUCTION

Keratin forms a major component of the epidermis and its appendages viz. hair, feathers, nails, horns, hoofs, scales and wool. On the basis of secondary structural confirmation, keratins have been classified into α (α-helix of hair and wool) and ß (ß-sheets of feather)[1] . The keratin fibrils in both the configurations are twisted in a parallel manner to form micro and macro fibrils that warrant stability to the fiber[2]. Keratins are also grouped into hard and soft keratins according to the sulfur content. Hard keratins found in appendages like feathers, hair, hoofs and nails have high disulfide bond content and are tough and inextensible. Whereas, soft keratins like skin and callus have low content of disulfide bonds and are more pliable[3]. Since most of the purified keratinases known to date cannot completely solubilize native keratin[4], their exact nature and uniqueness for keratinolysis is still an enigma in the world of proteases. Nonetheless, keratinases in nature have been continuously contributing to valorization of huge keratin containing wastes in the form of hair, feathers, dead birds and animals[5]. Keratinase and related products have many applications[6]. For example, the feather hydrolysates of *Bacillus licheniformis* PWD-1 and *Vibrio* sp. strain kr2[7] can be used as feed additives, while the keratinase from *Bacillus subtilis*S14 exhibits remarkable dehairing capabilities[8]. Keratinolytic activity has been reported for various bacteria, e.g. *Bacillus*[9]. Manczinger *et al.* (2003), Zerdani *et al.*, (2004) and Shih (1993) have reported keratinolytic bacteria are present in soil and poultry compost. The innovative aspect of the present work is to identify new sources of keratinases producing bacteria from poultry farm soil and this can have positive effect in solid waste management[10-12].

***Corresponding author.**
Dr. Deepak Kumar Malik
Assistant Professor
Department of Biotechnology Engineering
University Institute of engineering and Technology
Kurukshetra University, Kurukshetra, Haryana, India

MATERIALS AND METHODS

Isolation and screening of keratin degrading bacterial strains from poultry farm soil

The soil samples were collected from the feather dumping site near a poultry farm at Kurukshetra (Haryana), India. Soil samples was transferred in a sterile plastic bag and brought to Microbiology Lab, U.I.E.T., Kurukshetra University Kurukshetra, India for further processing. Isolation of bacterial strain was done by using enrichment technique (Martinus, 1901). 5 g soil is weigh and poured into the 250 ml flask containing 100 ml keratin media (10 gl^{-1} feather powder; 0.5 gl^{-1} NaCl; 0.3 $gl^{-1}K_2HPO_4$; 0.4 gl^{-1} KH_2PO_4; 0.1 gl^{-1} $MgSO_4.6H_2O$; pH 7.5). The flask was incubated for one week at 28 °C. The 10 ml culture broth from enriched flask was transferred into fresh keratin media containing feather as a sole carbon source up to 4 weeks. After enrichment isolation of bacteria was performed by serial dilution and plating

Table 1. Proteolytic activity of all isolated bacterial strains on skim milk agar plate

S.N	Bacterial isolate	Diameter of clear zone (cm) on 3rd day	Diameter of clear zone (cm) on 7th day	Diameter of clear zone (cm) on 14th day
1.	MKR1, MKR2, MKR3, MKR4, MKR6, MKR7, MKR8, MKR10, MKR12, MKR13, MKR14, MKR16, MKR18,MKR19,MKR20, MKR22	0	0	0
2.	MKR5	0	1	4.5
3.	MKR9	3	4.5	8
4.	MKR11	0	1	4
5.	MKR15	2	3	7.5
6.	MKR17	2	3.5	6.8
7.	MKR23	0	2	2
8.	MKR21, MKR24	0	1	1

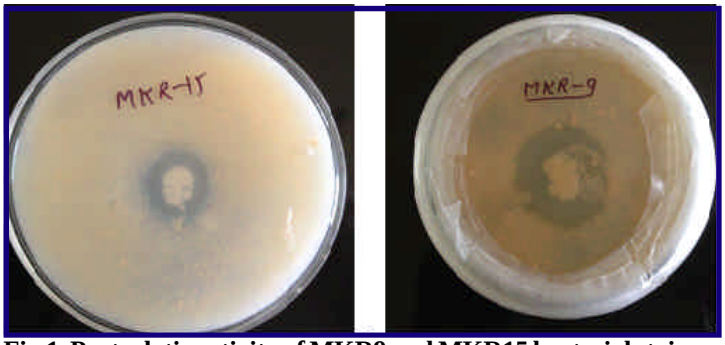

Fig.1. Proteolytic activity of MKR9 and MKR15 bacterial stain on skim milk agar plate

Fig.2. Keratinolytic degradation by isolated bacterial MKR15 and MKR9 in broth.

method on nutrient agar medium (NA). Plates were incubated at 37ºC for 24 to 48 hours. The bacterial isolates were further sub cultured on NA to obtain pure culture. Pure isolates were maintained in NA slants at 4ºC for further studies. The above isolated strains were streaked on skim milk agar plates for the testing of proteolytic activity. The plates were incubated at 37 °C for two weeks. Strains that produced clearing zones on these plates selected for keratinolytic activity. The bacterial isolates were inoculated in keratin medium in which feathers are the sole carbon source for the bacterial strain and incubated for two weeks.

RESULTS AND DISCUSSION

A total of 24 morphologically different bacterial strains were isolated by using enrichment technique from poultry farm soil samples. The isolated bacterial strains were streaked on skim milk agar plates for the testing of proteolytic activity. The plates were incubated at 37 °C for two week. Out of 24 only 8 isolate were showing proteolytic activity as indicated zone of clearance on skim milk agar plate as shown in Table1 and Fig 1. Out of these 8 proteolytic strains only 2 bacterial strains (MKR9 and MRR15) were showing significant keratin degradation ability by using chicken feather as a sole carbon source in keratin media and broth as shown in Fig 2. Finally bacterial isolate MKR9 which was showing complete degradation selected for further study. The isolated bacterial strain after further study can be used in degradation of feather waste. Isolated microbial strain can be used in leather industry for hair removal. Isolated microbial strain can in the formation of organic fertilizer after the degradation of feather waste.

REFERENCES

1. Akhtar, W. and Edwards H.G.M. (1997).Fourier-transform Raman spectroscopy of mammalian and avian keratotic biopolymers.SpectrochimActa 53: 81-90.
2. Kreplak, L., Doucet, J., Dumas, P. and Briki, F., (2004). New aspects of the á- helix to â-sheet transition in stretched hard á-keratin fibers. Biophys J.87:640–647.
3. Schrooyen, P.M.M., Dijkstra, P.J., Oberthu¨r, R.C., Bantjes, A.andFeijen, J. (2001) Partiallycarboxymethylated feather keratins. 2. Thermal and mechanical properties of films. J Agric Food Chem 49, 221–230.
4. Ramnani P, R Singh and R Gupta. 2005. Keratinolytic potential of *Bacillus licheniformis*RG1 : structural and biochemical mechanism of feather degradation. Can. J. Microbiol.51:191- 196.
5. Farag, A.M. and Hassan, M.A., (2004).Purification, characterization and Aimmobilization of a keratinase from *Aspergillusoryzae*. Enzyme Microb Technol.34: 85–93.
6. Gupta, R. and Ramnani, P., (2006). Microbial keratinase and their prospective application: an overview. *Appl*. MicrobiolBiotechnol.70(1): 21-33.
7. Grazziotin, A., Pimentel, F. A., de Jong, E.V. and Brandelli, A., (2006). Nutritional improvement of feather protein by treatment with microbial keratinase.Anim Feed SciTechnol.126(1-2): 135-144.
8. Macedo, A.J., da Silva, W.O.B., Gava, R., Driemeier, D., Henriques, J.A.P. and Termignoni, C., (2005). Novel keratinase from *Bacillus subtilis*S14 exhibiting remarkable dehairing capabilities.Appl. Environ Microbiol. 71(1): 594-596.
9. Williams, C.M., Richter, C.S., MacKenzie, J.M. and Shih, J.C.H.

(1990).Isolation, Identification and characterization of a feather-degrading bacterium.*Appl.Environ. Microbiol*.56: 1509-1515.

10. Manczinger L, Rozs M, VagvolgyiCS,Kevei F. *World Journal of Microbiology & Biotechnology.*2003, 19, 35–39.
11. Zerdani I, M Faid and AMalki. 2004. Feather wastes digestion by new isolated strains *Bacillus* sp. in Morocco. African J. of Bitechnol. 3(1):67-70
12. Shih, J.C.H. (1993).Recent development in poultry waste digestion and feather utilization.*A review of Poultry Sci*. 72:1617-1620.

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