IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
January 2017 Vol.:8, Issue:2

Screening of Keratinolytic Bacteria Form Keratin Waste Dumped Soil in Thanjavur (Dt), Tamil Nadu, India

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Tamilkani. P., Karnan.M., Kanimozhi.K and Panneerselvam.A.

PG and Research Department of Microbiology, A.V.V.M Sri Pushpam College (Autonomous), Poondi – 613503, Thanjavur (Dt), Tamil Nadu, India.

Submission: 2 January 2017
Accepted: 7 January 2017
Published: 25 January 2017

Keywords: Milk agar medium, Nutrient agar, Basal liquid medium, Keratin hydrolysis.

ABSTRACT

The aim of the study was to isolate Keratinolytic bacteria from feather dumping soil. Ten isolates were selected after growth on Nutrient agar and they named as TKP1to TKP10. All the ten isolates were subjected to primary screening on milk agar plates, among the ten isolates, four were showed proteolytic activity in terms of making clear zone surrounding their colony on the milk agar medium. The four positive isolates were again subjected to the secondary screening on feather broth and two isolates, TKP1 and TKP3 were showed degradation of feather during their growth. Though the degradation of process was taking long time, all these two achieved complete degradation of feather as the sole organic source for carbon, sulfur and energy. This novel Keratinolytic bacterial isolates have potential biotechnological use in processes involving keratin hydrolysis.

HUMAN JOURNALS
www.ijppr.humanjournals.com

INTRODUCTION

Keratin is the most abundant structural protein in skin, hair, wool and feathers. Keratins are proteins that form hard fibers and are components of epidermal and skeletal tissues. Disulfide and hydrogen bonds make keratin a stable protein resistance to proteolytic hydrolysis (Arai *et al*., 1996; Jones *et al*., 1999). Feather contains over 90% of crude protein in the form of keratin (Radhika Tatineni *et al*., 2008).

Feathers are almost pure keratin protein (90% or more), found as waste or byproducts at poultry processing plants. They reach millions of tons per year worldwide. Increasing quantities of feathers can lead to environmental pollution (Sanaa Tork *et al*., 2013).

Keratinase research has gained momentum because of its additional industrial and biotechnological applications. Other than those in the conventional sectors of proteases. (Gupta and Ramnani, 2007). *Keratinases* find application in feed industry, fertilizers, pharmaceutical sector and as dehairing enzyme in leather industry (Allpress *et al*., 2002; Anbu *et al*., 2005; Syed *et al*., 2009).

Keratinase which can degrade feather with dissolution of barbs and shafts in absence of any redox or pre-treatment will be ideal for prion degradation (Ekta Tiwas and Rani Gupta, 2010).

Keratin by virtue of its insolubility and resistance to proteolytic enzymes is not attacked by most of living organisms. Nevertheless, keratin does not accumulate in nature and, therefore, biological agencies can be presumed to accomplish its removal (Joshi *et al*., 2007).

They hydrolyse the keratin by synthesizing specific class of extracellular enzymes called *keratinases,* which degrade keratin into small peptides that can be utilized by the cell (Lucas *et al*., 2003).

The keratinolytic microorganisms and technologies developed for feather degradation not only remove the waste feathers efficiently from nature but also make the by-products of the process as a valuable protein supplement (Pushpalata *et al*., 2010).

The protein chains are packed tightly either in α-helix (α-keratins) or in β-sheet (β-keratins) structures, which fold into final 3-dimensional form (Kim, 2007; Esawy, 2007; Krelpak *et al*., 2004).

These proteins belonging to the scleropeptides group are compounds that are extremely resistant to the action of physical, chemical and biological agents. One of the main characteristics of keratins is that they have high mechanical stability and resistance to proteolytic degradation, which depends on the disulfide and hydrogen bonds, salt linkages and other cross linkings (Korkmaz *et al.*, 2004; Hoq *et al*., 2005).

A group of proteolytic enzymes which are able to hydrolyze insoluble keratins more efficiently than other proteases are called *keratinases* produced by some microorganisms. *Keratinase* properties depend upon its procedures. It is usually a serine protease. Keratinolytic enzymes have important utilities in biotechnological processes involving keratin-containing wastes from poultry and leather industries, through the development of non-polluting processes (Veslava Matikeviciene *et al.*, 2009).

Keratinases are inducible and different keratin-containing materials such as feathers, hair and wool can be used as substrates for *keratinase* production (Gupta and Ramnani, 2006). Feather was the mostly utilized substrate, while human hair was rarely utilized, especially by *Bacillus* sp. Another keratin-containing materials, silk as well as feathers and hair, are largely produced in China and these may also be potential substrates for *keratinase* production (Cheng-gang Cai, 2008).

The crude *keratinase* enzyme increased the digestibility of commercial feather meal and could replace as much as 7% of the dietary protein for growing chicks. Keratinolytic microorganisms and their enzymes may be used to enhance the digestibility of feather keratin. They may have important applications in processing keratin-containing wastes from poultry and leather industries through the development of nonpolluting methods (Saha and Dhanasekaran, 2009).

MATERIALS AND METHODS

Maintenance of bacterial isolates

The bacterial isolates were maintained at microbiology laboratory in A. V. V. M. Sri Pushpam College (Autonomous), Poondi, Thanjavur. The Nutrient agar medium (Composition (in g/l): Peptone – 5.0, Beef Extracts – 3.0, Agar – 15.0 and the pH was adjusted 7) was sterilized and poured into sterile petri dishes. Inoculation of suspected

bacterial isolates was done on solid medium surface and incubated the plates at room temperature for 18-24 hours.

Primary screening of keratinolytic bacteria (Saha and Dhanasekaran, 2009)

Milk agar medium (Composition (in g/l): Peptone – 5.0, Yeast Extract – 3.0, Dextrose – 1.0, Skim milk powder – 10.0, Agar – 15.0 and pH was maintained at 7.2) was used for the primary screening of keratinolytic bacteria (Riffel and Brandelli, 2006). All the ingredients of milk agar medium were sterilized in autoclave except Skim milk powder. Skim milk powder was added separately once the medium reached the tolerable temperature (45^0C) and poured the medium in sterile petri dishes. Suspected bacterial isolates, which already maintained in Nutrient Agar Medium, were inoculated in milk agar plates. The plates were incubated at room temperature for 48 hours and examined the plates for clear zone formation on the milk agar plate.

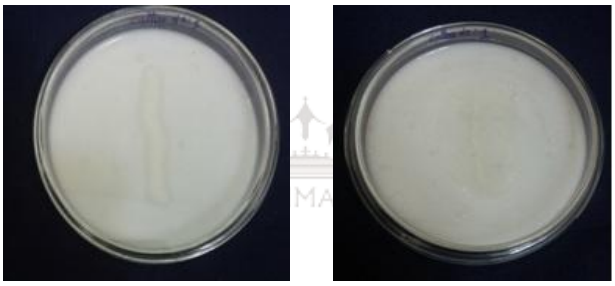
Secondary screening of keratinolytic bacteria (Saha and Dhanasekaran, 2009)

All positive isolates were obtained from the primary screening and subjected to perform the secondary screening in order to isolate the feather degrading bacteria. Modified Basal liquid medium (Composition in (g/l) : Mg SO_4.7 H_2O – 0.2 g/l, K_2HPO_4 - 0.3 g/l, K H_2PO_4 – 0.4 g/l, $CaCl_2$ – 0.22g/l and Yeast Extract – 0.1g/l) supplemented with raw chicken feather was used for the secondary screening (Mona,2008). Whole raw feather was collected from chicken processing shop. Feathers were washed properly with tap water to remove the blood and other dust particles from it and followed by washing with distilled water. Washed, cleaned, while chicken feather was dried at room temperature. Twenty five milliliters of modified basal liquid medium was taken in each boiling test tubes and added one cleaned, dried medium size chicken feather to the each boiling tubes. Sterilized the medium and inoculated the isolates once the medium got cool. Selected isolates were chosen based on their zone forming capability on the milk agar medium. Incubated the boiling tubes at room temperature and examined the tubes for 30 days.

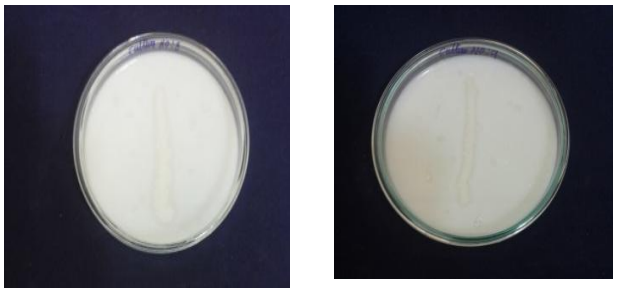
RESULTS AND DISCUSSION

Primary screening

In the present study, all the 10 isolates (*Bacillus cereus, Staphylococcus aureus, Microbacterium arborescens, Campylobacter jejuni, Listeria grayi, Corynebacterium striatum, Clostridium butyricum, Micrococcus luteus, Enterococcus faecium and Brachybacterium faecium*) were subjected to primary screening on milk agar plate among the 10 isolates TKP1, TKP3, TKP8 and TKP9 were formed the clear zone, which supported the degradation and utilization of casein (Skim milk powder) by the respective isolates. (Fig - 1).Saha and Dhanasekaran, (2010) stated that all the 22 isolates were subjected to primary screening on milk agar plate and among the 22 isolates SD4, 5, 6, 7, 8, 13, 14 and 15 were formed the clear zone, which supported the degradation and utilization of casein (skim milk powder) by the respective isolates.



TKP1 with clear zone TKP3 with clear zone



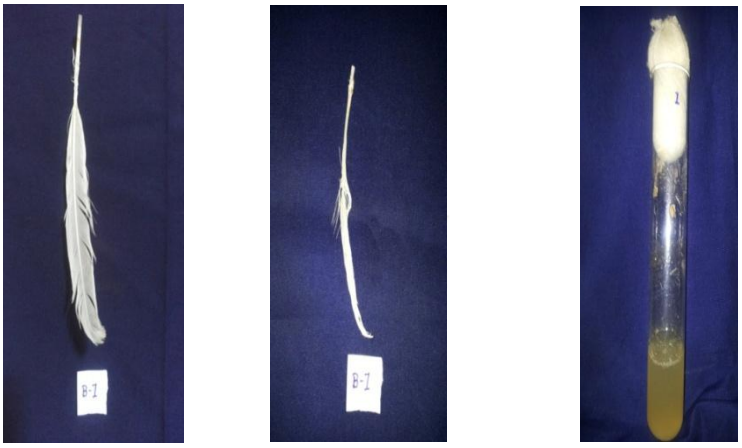
TKP8 with clear zone TKP9 with clear zone

Fig 1: Isolated bacterial colonies on Skim agar plate

Secondary screening

Saha and Dhanasekaran, 2010 reported that Among the positive isolates and SD5, 6 and 7 were able to degrade the feather among the 8 isolates selected through primary screening. All these three, SD5, SD6 and SD7 isolates were found to degrade the whole chicken feather in modified Basal liquid medium after 15-25 days of incubation period. Isolate, SD15 was observed with different character in the same modified Basal liquid medium that it grew on the feather surface but unable to degrade the feather.

In the secondary screening, among the 4 isolates, two strains TKP1 and TKP3 were able to degrade the feather effectively in modified basal liquid medium for after 15-25 days of incubation period. Hence these two strains were potent feather degrading strains from the present study.



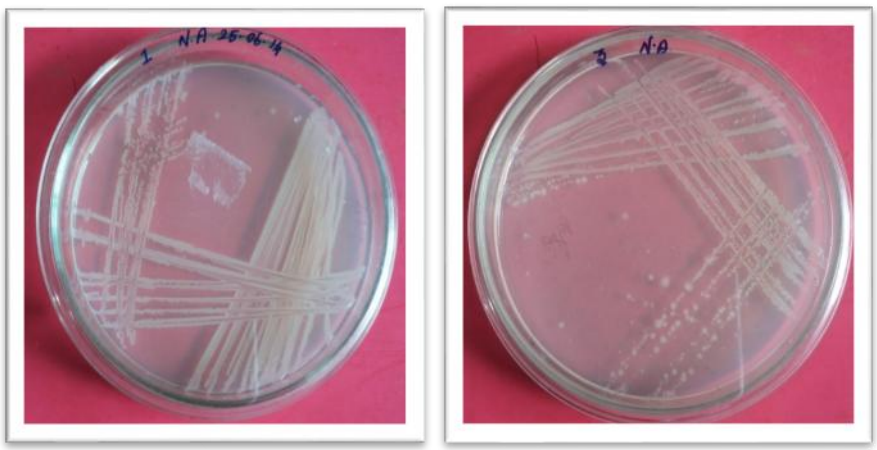
control **20 Days** **30 Days**

Fig 2: Degradation of feather by TKP1

control **20 Days** **30 Days**

Fig 3: Degradation of feather by TKP3

In the present investigation, bacteria were isolated from feather dumping soil that owned keratinolytic activity and ability to degrade the keratin waste. Preliminary screening test indicated that isolate TKP1, TKP3, TKP8 and TKP9 were capable of degrading and utilizing the casein which confirmed their proteolytic nature. Isolates grown on medium containing whole raw feather could utilize feather as a unique carbon and nitrogen source and secondary screening indicated TKP1 and TKP3 were the best two isolates among the other isolates capable of degrading. (Fig - 2 & 3).



TKP1 **TKP3**

Fig 4: TKP1 and TKP3 on Nutrient agar medium

Feather is rich in keratins and consists of high disulfide bonds, its make very hard to degrade the feather. Though TKP1 and TKP3 these two isolates took long time (15-25) for feather degradation, but they showed the significant property to degrade the feather that is difficult to achieve.

REFERENCES

1. All Press, J.D., Mountain, G and Gowland, P.C., 2002. Production, purification and characterization of an extracellular keratinase from lysobacter NCIMB 9497 Lett. *Appl. Microbiol*. 34: 337-343.
2. Anbu, P., Gopinathan, S.C.B., Hildaa, A., Lakshmi Priyab, T and Annaduraic, G., 2005. Purification and characterization of keratinase from poultry farm isolate *Scopulariopsis brevicaulis* and statistical optimization of enzymes activity. *Enzyme Microbiol. Technol*., 36: 639-647.
3. Arai, K., Naito, S., Dang, V.B., Nagasawa, N and Hirano, M., 1996. Cross linking structure of keratin. VI. Number, type and location of disulfide cross linkages in low-sulphur protein of woll fiber and their relation to permanent set. *J. Appl. Polym. Sci*., 60: 169-179.
4. Cheng-Gang Cai., 2008. Keratinase production and keratin degradation by a mutant strain of *Bacillus subtilis. J. Zhejiang. Univ. Sci. B.* 9(1) : 60-67.

5. Ekta Tiwary and Rani Gupta., 2010. Medium optimization for a novel 58 K Da dimeric keratinase from *Bacillus licheniformis* ER-15: Biochemical characterization and application in feather degradation and dehairing of hides. *Biosour. Technol.* 101: 6103-6110.
6. Esawy, M.A., 2007. Isolation and partial characterization of extracellular keratinase from a novel mesophilic *Streptomyces albus* AZA. *Res. J. Agri. Bio. Sci*, 3(6) : 808-817.
7. Gupta, R., and Ramnani, P., 2007. Microbial keratinases and their prospective applications. *Appl.Microbial.Biotechnol.* 70: 21-23.
8. Hoq, M.M., Siddiquee, K.A.Z., Kawasaki, H and Seki, T., 2005. Keratinolytic activity of some newly isolated *Bacillus* species. *J. Bio. Sci.*, 5(2): 193-200.
9. Jones, L.., Simon, M., Watts. N.R., Booy, F.P., Steven, A.C and Parry, D.A.D., 1999. Intermediates filament structure: hard α – keratin. *Bio. Phys. Chem*. 68: 83-93.
10. Joshi, S.G., Tejashwini, M.M., Revati, N., Shridevi, R and Roma, D., 2007. Isolation, Identification and Characterization of feather degrading bacterium. *Int. J. Poultry. Sci.* 6: 689-693.
11. Kim, J.D., 2007. Purification and characterization of a keratinase from a feather degrading fungus, *Aspergillus flavus* strain k-03. *Microbiol.*, 35: 219-225.
12. Korkmaz, H., Hur, H and Dincer, S., 2004. Characterization of alkaline keratinase of *Bacillus licheniformis* strain HK -1 from poultry waste. *Annals. Micribiol*. 54(2) : 201-211.
13. Krelpak, L., Doucet, J and Briki, F., 2004. New aspects of the α – helix to β – sheets transition in stretched hard α – keratin fibers. *Biophys. J.* 87: 640 – 647.
14. Lucas, F., Breennimann, O., Febbraro and Heeb, P., 2003. High diversity among feather degrading bacteria from a dry meadown soil. *Microbiol. Ecol*., 45: 282-290.
15. Mona, E.M., 2008. Feather degradation by a new keratinolytic *Streptomyces* sp. MS-2. *World. J. Microbiol. Biotechnol*., 24: 2331 – 2338.
16. Onifade, A.A., N.A.Al.Sane, A.A. Al – Musallan and S. Al-Zarban,1998. Potentials for biotechnological applications of keratin degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources. *Bioresour. Technol.*, 66: 169-179.
17. Pushpalata, S., Kainoor and Naik, G.R., 2010. Production and Characterization of feather degrading keratinase from *Bacillus* sp. JB 99. *Indian. J. Biotechnol*. 9: 384 – 390.
18. Rathika Tatineni., Kirankumar Doadapaneni., Rewi Chandra potumarthi.,Ravi Nagaraj Vellanki., Manjusha Thomas kandathil., Nilima Kolli and Lakshmi Narasu Mangamoori., 2008. Purification and characterization of an alkaline keratinase from *streptomyces*.sp. *Bioresour. Technol*. 99: 1596-1602.
19. Sanaa, E. Tork., Yasser E. Shahein., Amr E. El. Hakim., Azza, M., Abdel.Aty, Megda M and Aly.,2013. Production and characterization of thermostable metallo keratinase from newly isolated *Bacillus subtilis* NRC3. *Inter. J. Bio. Macromole.*, 55: 169-175.
20. Subhasish Saha and Dharmadurai Dhanasekaran, 2010. Isolation and screening of keratinolytic Actinobacteria form keratin waste Dumped soil in Tiruchirappalli and Nammakkal, Tamil Nadu, India. *Curr. Res. J. Bio.Sci*. 2(2): 124-131.
21. Syed, D.G., Lec, J.C., Li, W.J., Kim, C.J and Agasar, D., 2009. Production, Characterization and application of keratinase from *Streptomyces gulbargensis*. *Biores. Technol.*, 100: 1868-1871.
22. Veslava Matikeviciene., Danute Masiliuniene and Saulius Grigiskis, 2009. Degradation of keratin containing wastes by bacteria with keratinolytic activity. *Environ. Technol. Resour*.