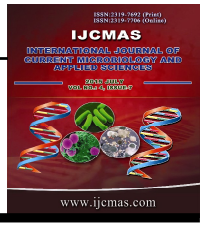
Int.J.Curr.Microbiol.App.Sci (2015) 4(7): 907-918

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 7 (2015) pp. 907-918**
http://www.ijcmas.com



Original Research Article

Detection of Keratinolytic Actinobacteria and evaluation of Bioprocess for Production of Alkaline Keratinase

Nandini Allure, Madhusudhan D.N. and Dayanand Agsar*

A-DBT Research Laboratory, Department of Microbiology,
Gulbarga University, Kalaburagi 585106, India
**Corresponding author*

A B S T R A C T

A large number of actinobctria were reported from the regional soil around poultry farms. Few isolates of actinobactria could reveal the keratinolytic activity and an actinobactrium DNA38 showed higher keratinolytic activity on starch feather meal agar. This potential isolate of actinobactrium DNA38 was identified as *Streptomyces* sp. based on morphological, biochemical and physiological properties, and further characterized as *Streptomyces minutiscleroticus* DNA38 by 16S rRNA analysis. Submerged bioprocess was found to be a suitable process for the production of higher amount (122.1 IU) of keratinase, when compared to solid state bioprocess with lesser amount (34.9 IU) of keratinase. High titer production of keratinase by *Streptomyces minutiscleroticus* DNA38 was achieved successfully under submerged bioprocess using chicken feathers as substrate, along with starch as source of carbon and few mineral salts. Alkaline pH (9.0) of the medium and higher incubation temperature (40 °C) reveals the production of thermophilic alkaline keratinase, which can be further explored for enhanced production aiming at myriad applications.

Keywords

Streptomyces; Molecular analysis; Submerged bioprocess; Solidstate bioprocess; Alkaline keratinase

Introduction

Keratin is an insoluble, high stable protein found mostly in feathers, wool, nails and hair of vertebrates (Shih, 1993). Keratin is resistant to the common proteolytic enzymes, papain, pepsin and trypsin (Papadopoulos et al., 1986). The high resistance of keratin to proteases may be attributed to the molecular conformation of their structural amino acids, that is tightly packed in the α-helices (hairs) and β-sheets (feather) in the presence of cystine disulfide bonds, hydrogen bonds and hydrophobic interactions (Parry and North, 1998) . The enzyme has received particular attention for its relevant applications in various types of agro and biotechnological industries. Certain microorganisms hydrolyze the keratin by synthesizing specific class of enzymes, which degrade keratin in to small peptides that can be utilized further by the cells. Several feather and hair degrading Streptomyces have been isolated from soil, poultry wastes, hair, debris and

animal skin. Keratinases, a group of serine metallo proteases, release the free amino acids from keratinous proteins. After treatment with keratinase, feather can be used as feeders, fertilizers and insoluble polymers (Yamauchi et al., 1996). Feathers consist of about 5-7% of total weight of mature chicken from poultry processing plants, approximated by about million tons produced annually, worldwide. Feather from the poultry processing plant is the common source for the accumulation of more than 90% of keratinous proteins in the environment, causing pollution (Onifade et al., 1998).A current value-added use for feathers is the conversion to feather meal, adigestible dietary protein for animal feed, using physical and chemical treatments. These methods can destroy certain aminoacids and decrease protein quality and digestibility (Moritz and Latshaw, 2001; Anbu et al., 2005). 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 non-polluting methods (Onifade et al., 1998). Keratinous wastes represent a source of valuable proteins and amino acids and could find application as a fodder additive for animals or source of nitrogen for plants.

Biodegradation by microorganisms possessing keratinolytic activity represents an alternative attractive method for improving thenutritional value of keratin wastes, as it offers cheap and mild reaction conditions for the production of valuable products (Kim et al., 2001). Streptomyces keratinases are of particular interest because of their action on insoluble keratin substrates and generally on a broad range of protein substrates. These enzymes have been studied for de-hairing processes in the leather industry.Despite all the work that has been done on production of proteolytic enzymes, relatively little information is available on keratinases. Upto now, a limited number of studies have been reported on the isolation of thermophiles, in particular thermophilic actinobacteria with the ability to hydrolyse feathers and other keratinous wastes. Enzyme production has been extensively studied by submerged and solid state bioprocesses. Submerged system is usually implemented in case of bacterial enzyme production, due to the requirement of higher water potential (Chahal, 1983). Solid state system is preferred when process require lesser water potential (Troller and Christian, 1978). The main reason can be attributed to the metabolic differences of water requirement. The metabolic differences of microorganisms involved in the submerged and solid state systems have a direct impact on the quantity of the product. Many actinobacteria are also reported to produce important commercially viable enzymes by either submerged or solid state bioprocesses. Normally, keratinase has been produced by submerged bioprocess, but in recent years, it is also being produced under solid state bioprocess. With regard to productivity aspect, it can be inferred that, both the systems are equally emerging popularly as field of choice for the production of keratinases. The aim of this study was to screen some actinobacteria for their ability to degrade native feather followed by identification of a new feather-degrading Streptomyces sp. and evaluation of suitable bioprocess for the production of keratinase.

Materials and Methods

Isolation and screening of keratinolytic actinobacteria

Unique ecological sites of poultry farms were identified for collection of soil samples around Kalaburagi region to isolate

actinobacteria. The soil samples were collected from the selected spots as per the standard procedures (Skinner, 1951). The top layer of the soil was removed for about 5 to 6 cm and with a clean spatula or scoop the soil was collected in sterile airtight polythene bags. The collected soil samples were enriched for isolating actinobacteria by different methods (Agate and Bhat, 1967; Pridham et al., 1956/57). One gram of soil suspension was added to 100ml of 1.4 % phenol solution and kept at room temperature for 10 minutes. The mixture was diluted further (Lawrence, 1956) and used to isolate actinobacteria by following standard serial dilution plate culture method (El-Nakeeb and Lechevalier, 1963). 0.1 ml of the sample from the respective dilutions was plated on starch casein agar, where in casein of SCA was replaced with chicken feather meal(Soluble Starch - 10.0 g; K_2HPO_4 - 2.0 g; KNO_3 - 2.0 g; NaCl - 2.0 g; chicken feather meal - 5 g; $MgSO_4$ - 0.05 g; $CaCO_3$ - 0.02 g; $FeSO_4.H_2O$ - 0.01 g; Agar - 20 g; Distilled water - 1000 ml; pH - 8.5). The inoculated plates were incubated at 40 ºC for 120 h. Based on the growth on feather medium, the actinomycete colonies were selected and sub-cultured on the skimmed milk agar (Ronald, 2010). Based on zone of hydrolysis on skimmed milk agar the isolates were selected and assessed for the keratinolytic activity using starch feather meal agar by plate culture method. Selected potential isolates of actinobacteria were identified up to the level of genus by morphological (Shirling and Gottlieb, 1976), biochemical and physiological properties (Williams et al., 1989).

Molecular characterization of keratinolytic Streptomyces

Chromosomal DNA was extracted by using Chelex 100 (Sigma-Aldrich, USA) chelating ion exchange resin method (Laurent et al, 1999). Around 100 nanogram DNA was used for amplification of 16S rRNA gene. 16S rRNA amplification of cultures was done using universal F27 (5'AGAGTTTGATCMTGGCTCAG-) and R1525 (5'TACGG(C/T)TACCTTGTT ACGACTT) primer (Weisburg et al, 1991). PCR master mix was prepared containing 100ng (1µL) DNA, 1.25 µL of both primers F27 and R1525, 2.5 µL each of 10X PCR buffer and 1mM dNTPs, 0.25 µL Taq polymerase and 17 µL nuclease free PCR grade water to make up a volume of 25 µL. PCR master mix is a premixed ready to use solution containing Taq DNA polymerase, reaction buffers containing MgCl2, dNTPs at ideal concentrations for efficient amplification of templates by PCR. The program was set with the initiation temperature of 94°C for 3 minute, followed by 34 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 1.30 minute. The final extension after these 34 cycles is at 72°C for 7 minute followed by hold at 4°C. Accuracy of PCR product was visualized on agarose gel. The PCR product was purified using Genei pure™ Quick PCR Purification Kit and were then sequenced using a Big Dye Terminator kit, version 3.1, on an automatic ABI 3100 sequencer (Applied Biosystems Inc.). The Sequences were compared using 16S rRNA gene sequences from EzTaxon to restore closest relatives. The entire work of molecular characterization was facilitated at Genomics Services Xcelris Labs Ltd, Ahmedabad.

Evaluation of bioprocess for production of keratinase

Submerged and solid state bioprocesses were evaluated in empirical conditions for the production of keratinase by the potential isolate of Streptomyces DNA38, explained in brief as follows.

Submerged bioprocess: The bioprocess

(Lerch and Ettlinger *et al*., 1972) was carried out using StreptomycesDNA38 in a 250 ml Erlenmeyer flask containing 100 ml of starch feather medium with pH 9.0. After sterilization of the medium at 121 ºC for 15 min, 1 ml suspension of three days old test isolate with spore count $1x10^8$ spores/ml was inoculated and kept for incubation at 40 ºC for week in shaker incubator at 180 rpm. The amount of keratinase produced was estimated at every 24 h.

Solidstate bioprocess: 25 g of substrate (chicken feathers) was taken in 250 ml Erlenmeyer flask and rehydrated with mineral salt solution (K_2HPO_4 - 2.0 g; KNO_3 - 2.0 g; NaCl - 2.0 g; chicken feathers - 5 g; $MgSO_4$ - 0.05 g; $CaCO_3$ - 0.02 g; $FeSO_4.H_2O$ - 0.01 g; Distilled water - 1000 ml; pH –9.0) to achieve 65% moisture content. Initial pH was adjusted to 8.0. The cotton plugged flasks were autoclaved at 121ºC for 15 min and allowed to cool at room temperature. The contents of flask were inoculated with 1 ml of spore inoculum (spore count $1x10^8$ spores/ml), mixed gently and incubated in a slant position at 40 ºC in a humidity chamber at 65-70% relative humidity. The substrates were analyzed for the production of keratinase at an interval of every24 h.

Estimation of keratinase

The keratinase activity of the fermented broth or extract of both the bioprocess was estimated by modified method of Cheng *et al*. (1995) using keratin as a substrate. The reaction mixture containing 1ml of 1% keratin in phosphate buffer (pH 8.0) and 0.5 ml of fermented broth or extract was incubated at 30 ºC for 30 min. After incubation, the reaction was terminated by adding 2 ml of 10% trichloroacetic acid (TCA). After the separation of untreated keratin as pellet by centrifugation, 1ml of clear supernatant was mixed with 5ml of 0.4 M Na $2CO_3$ and 0.5 ml of Folin-Ciocalteau's phenol reagent. The absorbance was measured at 660 nm against blank after 30 min. All assays were carried out in triplicate. One unit of keratinase activity was defined as the amount of enzyme that released one microgram of tyrosine per minute under the standard assay conditions.

Results and Discussion

Isolation and Screening of Actinobacteria

More than hundred colonies of actinobacteria were observed on the plates of starch feather meal agar with typical colony characters (Figure 1). Forty two colonies of actinobacteria were selected randomly based on prominent colony characters and screened for the synthesis of protease on skimmed milk agar. Among forty two isolates, five isolates formed the zone of catalysis (Figure 2) indicating the production of protease. The four prominent proteolytic isolates of actinobacteria were further inoculated on the starch feather meal agar and incubated. Based on keratinolytic zone, the isolate DNA38 was emerged as a potential strain for the synthesis of kertainase (Figure 3).

Potential isolate of keratinolytic actinobacterium on starch feather meal agar isolated from the soils from surroundings of poultry farm wasidentified based on standard colony characters and microscopic features (Table 1). Pigmentation pattern (Shirling and Gottlieb, 1976)of aerial/substrate mycelium and diffusible pigment is an important attribute for the identification of anactinobacterium. Gram's positive property, high mycelial branching and sporulation feature confirms the isolate of actinobacterium as belonging to the genus Streptomyces. Important biochemical properties such as catalase production, no H_2S production and nitrate reduction were

also recorded (Williams et al., 1989). The growth of actinobacterium at higher range of temperature, sodium chloride and pH were assessed for the better understanding of its physiological adaptability and tolerance. Several isolates of actinobacteria were reported (Shiveerakumar et al., 2013; Madhusudhan et al., 2014; Mazhari et al., 2014) earlier from the regional alkaline soils for the production of various enzymes at our A-DBT (Actinobacteria- Diversity and Bioprocess Technology) research laboratory and explored for various biotechnological applications.

Molecular characterization of Streptomyces DNA38

Actinobacteria can be analyzed at various molecular levels to gain information suitable for constructing databases and effective identification. Sequence analysis of various genes provides a stable classification and accurate identification, which has become the corner stone of modern phylogenetic taxonomy (Muyzer et al., 1996). The region of 16S rRNA gene are highly variable and differ significantly between species where as other areas are more conserved and suitable for identification at the generic level (Amann and Ludwig, 2000).

Apartial 16S rRNA gene sequence of isolate DNA38 (566 nucleotides) was determined (Genbank, NCBI Accession number: KP419934). A phylogenetic tree was constructed based on 16S rRNA gene sequence to show the comparative relationship between isolate DNA38 and other related Streptomyces species (Figure 4). The comparative analysis of 16S rRNA gene sequence and phylogenetic relationship reveals that isolate DNA38 lies in a subclade with *Streptomyces minutiscleroticus*, sharing 99.7 % of 16S rRNA gene sequence similarity.

Bioprocess for the production of keratinase

The efficient isolate *Streptomyces minutiscleroticus* DNA38was considered for the quantitative production of keratinase under submerged bioprocess using starch chicken feathers broth and solidstate bioprocess under chicken feathers mineral salt substrate over a period of 168 h and 192 h respectively. The isolate DNA38 showed 122.1 IU of keratinase activity (Figure 5) under submerged process and 34.9 IU of keratinase activity (Figure 6)under solid state process at 144 h and at 168 h of incubation respectively. Quantitative estimation for the production of keratinase is most important criteria to select the suitable bioprocess for the optimized enhanced production of keratinase.

Production of keratinase has been achieved in liquid cultures of various microorganisms and least from fungi. Bacteria like *Microbacterium* sp.- 10.5 U/mL (Riffel Alessandro and Adriano Brandelli 2006), *Pseudomonas aurogenosa*- 35.25 U/mL (Li Jung Yin et al., 2006), *Bacillus* sp. JB99 - 35.0 µg/mL/min (Pushpalata and Naik – 2010), *B. subtilis*- 463 U/mL (Ana Maria Mazotto 2011), *Bacillus* sp. - 10 KU/mL (Jeevana Lakshmi et al., 2013) have been reported for the production of keratinase. Among Actinobacteria, Streptomyces sp. MS-2 - 9.11 U/ml (Mona E. M. Mabrouk 2008), *Streptomyces gulbargensis*– 1.39 U/mL (Dastager et al., 2009), *Streptomyces albogriseolus* NGP 71.43 U/mL (Selvam et al., 2013) and *Saccharothrix xinjiangensis*– 92.81 U/mL (Shilpa Ashok Jani et al., 2014)and many more were reported for the synthesis of keratinase. Several investigators have reported on the occurrence of a variety of keratiophilic fungi including dermatophytes in soils of varying habitats.

The predominant keratinophilic fungi reported in most studies include *Chrysosporium* spp (mainly *C. indicum*, *C. tropicum* and *C. keratiophilum*) and the dermatophyte *M. gypseum* (Hoog et al., 2000). Production of keratinase have also been achieved in solid state culture system. Fungi are the favorite for the production of keratinase under solid state bioprocess followed by bacteria. Reports on the production of keratinase under solid state bioprocess using actinobacteria are very scanty. Mervat et al. (2010) reported the production of keratinase using Aspergillus niger (160 U/mg) under solid state bioprocess and maximum of 172.7 U/mL of keratinase activity was achieved by Ana Maria et al. (2013) using Endophytic *Penicillium* sp. *Bacillus pumilus* GHD could able to produce 73 U/mg of keratinase under solid state bioprocess using sugar cane bagasse as substrate (Ghada et al., 2011). The present investigation reveals relatively a higher amount keratinase production by an efficient isolate of an actinobacterium, *Streptomyces minutiscleroticus* DNA38, under submerged bioprocess with 9.0 pH of the medium. Thus produced alkaline protease can be explored further for various applications.

Table.1 Morphological, biochemical and physiological characters of actinobacterium

Characters	DNA38
Colony	
Aerial mycelium	Grey
Substrate mycelium	White
Microscopic	
Gram's staining	+ ve
Mycelium branching	Poor
Sporulation level	Scanty
Biochemical	
Catalase production	- ve
H_2S production	+ ve
Nitrate reduction	+ ve
***Growth at Temperature**	
$40^0/45^0/50^0$ C	++ / + / +
Sodium chloride	
1 % / 2% / 3 %	++ / ++ / +
pH	
8.0 / 9.0 / 10.0	++ /+++ / ++

*+: Poor growth, ++: Moderate growth, +++: Maximum growth

Figure.1 A representative plate showing colonies of actinobacteria on starch casein agar

Rear View **Front View**

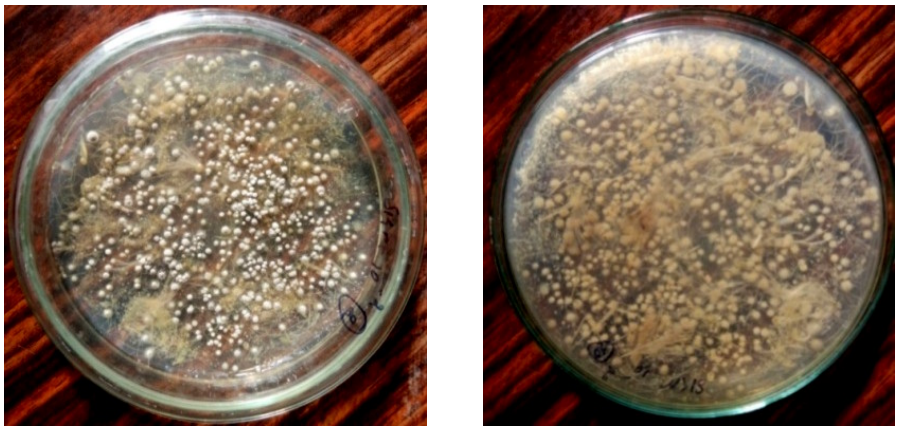


Figure.2 Zone of catalysis by actinobacteria on skimmed milk agar

Rear View **Front View**

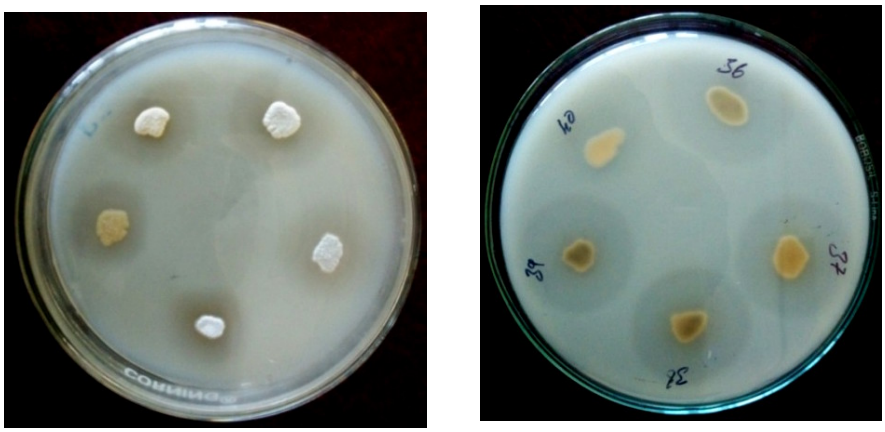


Figure.3 Keratinolytic zone by prominent isolates of actinobacteria on starch feather meal agar

Rear View **Front View**

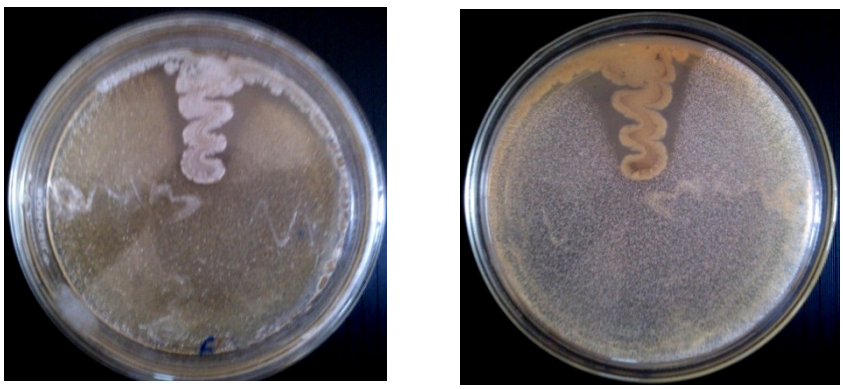


Figure.4 Phylogenetic tree indicating the systematic position of Streptomyces DNA38

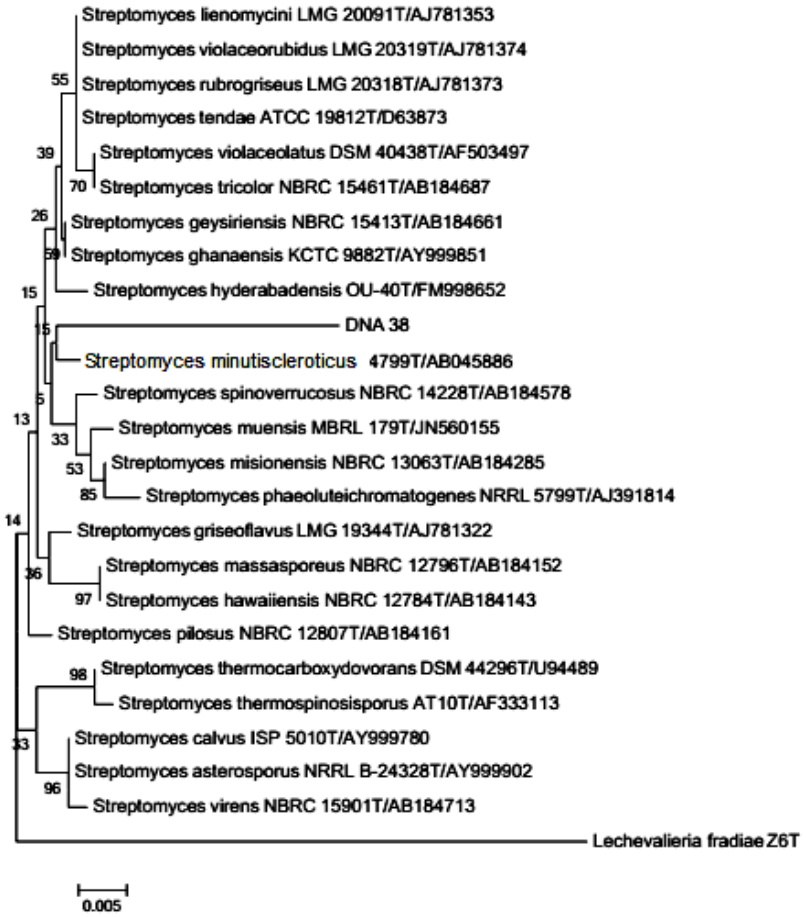


Figure.5 Keratinase produced under submerged bioprocess using chicken feathers in starch and mineral brothby *Streptomyces minutiscleroticus* DNA38

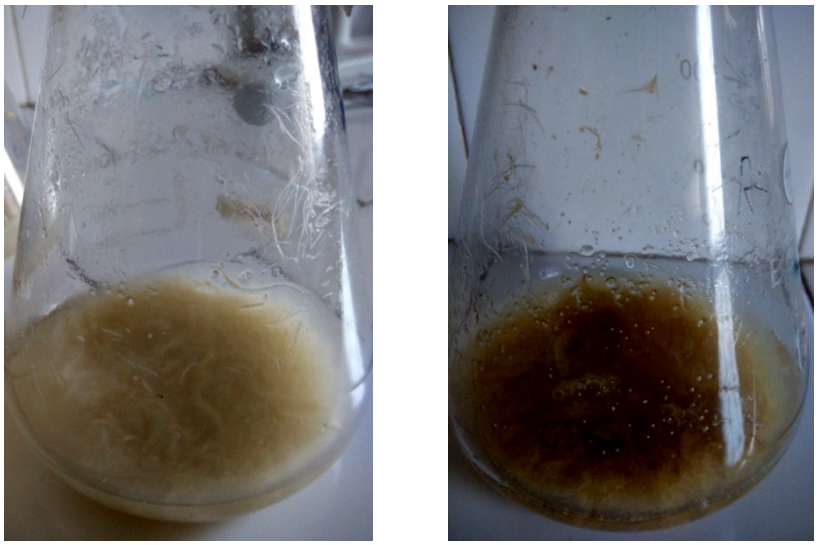


Figure.6 Keratinase produced under solid state bioprocess on chicken feathers with starch and mineral broth as moisture by *Streptomyces minutiscleroticus* DNA38

The regional soil samples surrounding the poultry farms exhibited a rich occurrence of actinobacteria. Few colonies of actinobacteria obtained from the crowded plate showed protease activity on skimmed milk agar and one isolate could reveal maximum keratinolytic activity on starch feather meal agar. The most efficient keratinolytic actinobacterium was characterized as *Streptomyces minutiscleroticus* DNA38 based on morphological characters, biochemical properties, physiological features and molecular charactterization by 16S rRNA analysis. Maximum of 122.1 IU and 34.9 IU keratinase activity was achieved by *Streptomyces minutiscleroticus* DNA38 under submerged and solid state bioprocesses respectively. Production of alkaline keratinase at pH 9.0 of the medium under submerged bioprocess at 40 °C is the striking feature of the present investigation. Submerged bioprocess can be further explored for the enhanced production of alkaline keratinase aiming at various biotechnological applications.

References

Agate, A.D. and Bhat, J.V. 1967. Increase in actinomycetal population of stored soils.
Curr. Sci. 36: 152-153.

Amann, R. and Ludwig, W. 2000. Ribosomal RNA-targeted nucleic acid probes for studies in microbial ecology. FEMS Microbiology Reviews. 24(5): 555–565.

Ana Maria Mazotto, Rosalie Reed Rodrigues Coelho, Sabrina Martins Lage Cedrola, et al., 2011.Keratinase Production by Three *Bacillus* spp. Using Feather Meal and Whole Feather as Substrate in a Submerged Fermentation. Enzyme Research. Article ID 523780, 7 pages. doi:10.4061/2011/523780.

Ana Maria Mazotto, Sonia Couri, Mônica, C.T., Damaso and Alane Beatriz Vermelho. 2013. Degradation of feather waste by *Aspergillus niger* keratinases: Comparison of submerged and solid-state fermentation. International

Biodeterioration & Biodegradation. 85: 189-195.

Anbu, P., Gopinath, S.C.B., Hilda, A., Lakshmi Priya T. and Annadurai, G. 2005. Purification of keratinase from poultry farm isolate - *Scopulariopsis brevicaulis* and statistical optimization of enzyme activity. Enzyme Microb. Technol., 36: 639-647.

Chahal, D.S. 1983. American Chemical Society Symposium. American Chemical Society. Washington DC. 207: 421.

Cheng, S.W., H.M. Hu, S.W. Shen, H.Takagi, M. Asano, and Y.C. Tsai. 1995. Production and characterization of keratinase of a feather- degrading Bacillus licheniformis PWD-1. Biosci. Biotechnol. Biochem. 59: 2239-2243.

Dastager, G.S., Chan, Lee J., Wen – Jun, L., Chang – Jin, K. and Dayanand, A. 2009. Production, characterization and application of keratinase from *Streptomyces gulbargensis*. Bioresour. Technol. 100:1868-1871.

El-Nakeeb, M.A. and Lechevalier, H.A. 1963. Selective isolation of aerobic actinomycetes, Appl. Microbiol. 11: 75-77.

Ghada, E.A., Awad & Mona A., Esawy & Walla A., Salam & Bassem M., Salama & Amal F., Abdelkader & Ahmed El-diwany. 2011. Keratinase production by Bacillus pumilus GHD in solid-state fermentation using sugar cane bagasse: optimization of culture conditions using a Box-Behnken experimental design. Ann. Microbiol. 61:663–672.

Hoog, G.S., Guarro, J., Gene, J.*and*Figueras, M.J.2000.Atlas of Clinical Fungi, 2nd edn. *pp. 769. Utrecht:Centraalbureau voor Schimmelcultures.*

Jeevana Lakshmi, P., Kumari Chitturi, M. and Lakshmi, V.V. 2013. Efficient Degradation of Feather by Keratinase Producing Bacillus sp. International Journal of Microbiology. Article ID 608321. doi:10.1155/2013/608321.

Kim, J.M., Lim, W.J. and Suh, H.J. 2001. Feather-degrading *Bacillus* species from poultry waste. *Process Biochemistry*. 37(3): 287-291.

Laurent, F.J., Provost, F. and Boiron, P. 1999. Rapid identifiation of clinically relevant Nocardia species to genus level by 16S rRNA gene PCR. Journal of Clinical Microbiology. 37(1): 99–102.

Lawrence, L.H. 1956. A method of isolating actinomycetes from potato tissue and soil with minimal contamination. Can. J. Bot. 34: 44-47.

Lerch Konard and Ettlinger Leopold. 1972. Purification and characterization of a tyrosinase from *Streptomyces glaucescens.* Eur. J. Biochem. 31: 427-437.

Li-Jung Yin, Jein-Hwa, Lee and Shann-Tzong, Jiang. 2006. Isolation of a Keratinase-Producing Bacterium and purification of its keratinase. J. Fish. Soc. Taiwan. 33(4): 377-390.

Madhusudhan, D.N., Mazhari, B.B.Z., Dastager, S.G. and Agsar D. 2014. Production and Cytotoxicity of E xtracellular Insoluble and Droplets of Soluble Melanin by *Streptomyces lusitanus* DMZ-3. Bio. Med. Res. Int.

Mazhari Zainab, Bi Bi, Madhusudhan, D.N., Raghavendra, H., Dayanand Agsar and Syed Dastager. 2014. Development of bioconjugate from Streptomyces tyrosinase and gold nanoparticles for rapid detection of phenol constituents. Indian Journal

of Experimental Biology. 24.

Mervat Morsy El-Gendy. 2010. Keratinase production by endophytic Penicillium spp. Morsy1 under solid-state fermentation using rice straw. Applied Biochemistry Biotechnology. 162: 780–794.

Mona E. M. Mabrouk. 2008. Feather degradation by a new keratinolytic Streptomyces sp. MS-2. World J. Microbiol Biotechnol. 24: 2331–2338.

Moritz, J.S. and Latshaw, J.D. 2001. Indicator of nutritional value of hydrolysed feathers.Poultry Sci, 80: 79-86.

Muyzer, G., Hottentrager, S., Teske, A. and Wawer, C. 1996. Denaturing gradient gel electrophoresis of PCR-amplified 16S rDNA a new molecular approach to analyze the genetic diversity of mixed microbial communities. Molecular Microbial Ecology Manual. A.D.L. Akkermans, J.D. van Elsas, and F.J. de Bruijn, Eds. Kluwer Academic Publishers, Dordrecht. Th Netherlands. pp. 1–23.

Onifade, A.A., Al-Sane N.A., Al-Musallam, A.A. and Al-Zarban, S. 1998. Potentials for biotechnology applications of keratin degrading microorganisms and their enzymes for nutritional improvements of feathers and other keratins as livestock feed resources. Bioresour. Technol. 66: 1-11.

Papadopoulos, M.C., El-Boushy, A.R., Roodbeen, A.E. and Katelaar, E.H. 1986. Effects of processing time and moisture content on the amino acid composition and nitrogen characteristics of feather meal. Anim. Feed Sci. Technol. 14: 279-290.

Parry, D.A.D. and North, A.C.T., 1998. Hard α-keratin intermediate filament chains: Substructure of the N-and C-terminal domains and the predicted structure and function of the C-terminal domain soft type I and type II chains. J. Structure Biol. 122: 67-75.

Pridham, T.G., Anderson, P., Foley, C., Lindenfelser, H.A., Hesseltine C.W. and Benedict R.G. 1956/57. A section of media for maintenance and taxonomic study of Streptomyces. Antibiotics Ann. 947-953.

Pushpalata, S.K. and Naik, G.R. 2010. Production and characterization of feather degrading keratinase from Bacillus sp. JB 99. Indian Journal of Biotechnology. 9: 384-390.

Riffel Alessandro, and Brandelli Adriano. 2006. Keratinolytic bacteria isolated from feather waste. Brazil J. Microbiol. 37: 395-9.

Ronald Atlas M. 1947. Handbook of microbiological media, 4th Edition, CRC press, USA, 1862-1863.

Selvam, K., Vishnupriya, B. and Yamuna, M. 2013. Isolation and description of keratinase producing marine actinobacteria from South Indian Coastal Region. African Journal of Biotechnology. 12(1): 19-26.

Shih, J.C.H. 1993. Recent development in poultry waste and feather utilization – a review. Poultry Science. 72: 1617–1620.

Shilpa Ashok Jani, Raval Heta, Harnisha Patel, Drashti Darji, Ankit Rathod and Seema Pal. 2015. Production and characterization of keratinolytic protease from Streptomyces sp. Int. J. Curr. Microbiol. App. Sci. 4(5): 962-975.

Shirling, E.B. and Gottlieb, D. 1976. Retrospective evaluation of International Streptomyces Project

taxonomis criteria. In: Arai. T (eds.), Actinomycetes-the boundary microorganisms, Baltimore University, Park Press, London, Tokyo, pp. 9-41.

Shivaveerakumar, S., Madhusudhan D.N., Raghavendra, H. and Dayanand Agsar. 2014. Screening of Streptomyces and process optimization for the production of tyrosinase. J. Pure and Applied Microbiology. 8.

Skinner, F.A. 1951. A method for distinguishing between viable spores and mycelial fragments of actinomycetes in soils. J. Gen. Microbiol. 5:159–166.

Troller, J. A., and Christian, J.H.B. 1978. Water activity and food New York: Academic Press.

Weisburg, W.G., Barns, S.M., Pelletier, D.A. and Lane, D. J. 1991. 16S ribosomal DNA amplifiation for phylogenetic study. Journal of Bacteriology. 173(2): 697–703.

Williams, S. T., Goodfellow, M. and Alderson, G. 1989. Genus *Streptomyces* Waksman and Henrici 1943, 339. In: Bergey's Manual of Systematic Bacteriology, Eds: Williams, S.T., Sharpe, M.E., Holt, J.G., Williams and Wilkins, Baltimore, 4: 2452-2492.

Yamauchi, K., Yamauchi, A., Kusunoki, T., Khoda, A. and Konishi ,Y. 1996. Preparation of stable aqueous solutions of keratins and physiological and biodegradational properties films. J. Biomat. Mat. Res. 31: 439-444.