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NOCARDIOPSIS ALBA DMS 43377:A NOBLE POTENT FEATHER DEGRADING ACTINOBACTERIA ISOLATED FROM FEATHER WASTE IN TAMILNADU, INDIA

SUNEETHA V*, RAMAN KUMAR, NAVNEET NIMESH
Instrumental and food analysis laboratory, Chennai
***Corresponding author: E.Mail:vsuneetha@vit.ac.in; kumar.raman301189@gmail.com; Contact: 8124014747**

ABSTRACT

Keratin is a protein which is very hard and fibrous in nature. It can pose a great environmental threat as it is not digestible by simple protease enzymes. The major sources of keratinous wastes are in the form of feathers, horns, nails, claws and hairs. Therefore, microorganisms which would efficiently degrade and recycle such wastes are very much needed. A feather degrading geophilic, *Nocardiopsis alb*a DMS 43377 isolated from the soil sample collected from chicken feather dumped area using a baiting technique was capable of producing keratinase enzyme. The crude keratinase enzyme produced by actinomycetes was capable of degrading chicken feathers in an efficient manner as compared to the bacillus sp. The end product of partially degraded chicken feathers was protein containing different amino acids and may have potential application as an animal feed and in cosmetics. Thus, *Nocardiopsis alba* DMS 43377 could serve as a novel microbes to produce keratinase enzyme that can degrade the feathers of chicken quite efficiently.

KEY WORDS *Nocardiopsis Alba, Chicken feathers, Keratinase, Geophilic.*

INTRODUCTION

Feathers and human hairs are produced in large amount as a waste from poultry farm and tonsuring areas respectively. Feathers and hairs are composed of keratin protein which is fibrous, hard and insoluble in nature. Keratin protein is extensively crossed linked with disulphide bond, hydrogen bond and hydrophobic bond, resulting in mechanical stability and resistance to common proteolytic enzyme like trypsin, papain and pepsin. The feathers and hairs are rich source of protein and can be used as a feather meal for the protein supplement. The method used earlier was physical and chemical treatment to make the feathers as valuable feather meal product but these methods can destroy the quality as well as quantity of certain amino acids and proteins (Moritz and Latshaw, 2001). Feather degradation through keratinase producing microbes has been proved to be an efficient process for bioconversion, nutritional enhancement and eco-friendliness. Enzymatic method is extremely useful to convert the feathers into rare amino acids like serine, cysteine, threonine and methionine. Keratinolytic enzymes are produced by different microbes found in the soil such as Actinomycetes like Streptomyces (Syed, 2009) bacteria such as bacillus (Ramani and Gupta, 2004), Ferbidoacterium islandicum (Nam, 1938). Most of these microorganisms are isolated from poultry farm and related to the soil. We have isolated a new strain identified as *Nocardiopsis Alba* strain DSM 43377 from soil sample collected from poultry farm dumping area.

MATERIALS AND METHODS

Microorganism: Microbes were isolated from the soil where hairs and birds feather was dumped. This isolates were identified as actinomycetes species by doing different biochemical test and further analysed by analytical instruments.

Growth conditions: The microbes were grown at an optimum condition using nutrient agar (HI-VEG MEDIA).The strain was cultivated at room temperature in salt medium. Bird's feather and hairs were used as carbon and nitrogen source.

Feather collection and pre-treatment of feather: Feathers and hairs were collected from the poultry farm and tonsuring areas in and around Vellore shop respectively. The feathers were rinsed with water thrice followed by drying in the sun light for 24hrs. Thereafter, the feathers were cut in smaller pieces 2-3 centimetre and treated with the methanol and chloroform in the ratio of 3:1 to make the keratin substrate more soluble and vulnerable that can be easily degraded by the keratinase producing microbes. The obtained feathers were kept for 18 hours of Incubation period. Similar process repeated several times to enrich the substrate used in the experiment.

Collection of soil sample: 2 soil samples were collected from poultry and tonsuring areas in and around Vellore. These samples were tested for the presence of micro- organisms having the ability to grow and show keratinase activity. Tenfold serial dilution was made by mixing 1 gram of sample in the first test tube and then transferred 1 ml to the second test tube and so on.

Enzyme assays: The keratinolytic activity was measured by using protocol of Anson (1938) and keratin used as a substrate. The reaction mixtures contains 5mg of substrate and 0.8ml of Tris-HCl buffer (pH=7.5) and 0.2ml of culture filtrates and it was incubated for 30min at 50°c. The reaction was stopped by adding 1ml, 0.1M Tris-acetic acid (TCA). The mixture was filtered by watt man paper and 0.5ml of filtrate added with 1ml of ninhydrin. The filtrate was measured at 520nm for liberation of amino acid. The quantity was determined from standard tyrosine solution (50-500µgml^{-1}).

Protein determination: 2ml of reaction mixture was composed of 1ml of 2%casein in 0.2M of Tris-HCL, pH7.5 and 1ml of appropriately diluted enzyme. The reaction mixture was incubated for 30min in the water bath at 37°C. 2ml of 20% TCA was then added and centrifuged at 5000rpm for 15min. Enzyme was added to process the control after incubation and TCA was immediately added. The protein content of enzyme was determined by using Lowry et al. (1951).

Protease activity: Protease activity was determined using casein as substrate according to the method reported by Gessesse et al. (2003).The reaction mixture in a total volume of 2ml was composed of 1ml of 2% casein in 0.2M of Tris-HCl, pH7.5 and 1ml of appropriately diluted enzyme. The reaction mixture was incubated at 40°C for 30 min in water bath. 20% TCA of 2ml was added and the mixture was centrifuged at 5000rpm for 15min. A control was processed by adding enzyme to the mixture after incubation and TCA was immediately added.

Physiological properties of actinomycetes:

Effect of pH: Modified Bennett broth was sterilized, prepared and pH adjusted to 5, 7 and 8.5 using 0.1N HCL and NaOH. The tubes were incubated at 37°C for 7 days and 14days after incubation growth was recorded (Ivanko and Varvanets, 2004).

Effect of temperature: Modified Bennett broth was prepared and sterilized and the actinomycetes culture were incubated in the broth. The tubes were incubated at 37°C and 40°C for 7 to 14 days, then 4°C and 10°C respectively. After incubation growth was recorded. The pH and temperature were 7.9 and 43°C (Igantova, 1999).

RESULTS AND DISCUSSION

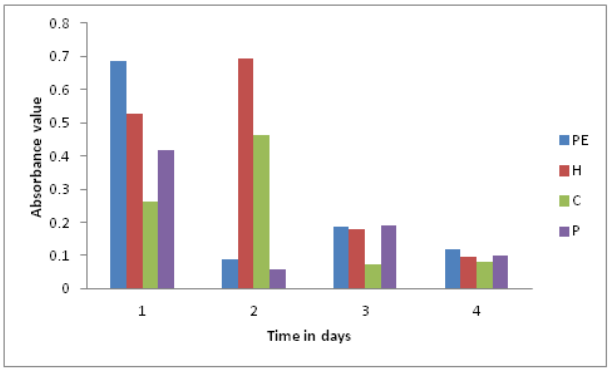

Figure.1. The graph plotted above between the time in (days) and absorbance value at 580 nm. The protease activity depleted with the time. Our sample PE and H has highest protease activity. Hence the OD value is high in these two samples compared to other. The depleted OD value with time showed that the produced amino acid used by the microbes for their growth. The obtained OD value showed higher than the OD value taken from standard amino acid.
PE (Peacock), P (Pigeon), H (Hen), C (Chicken)

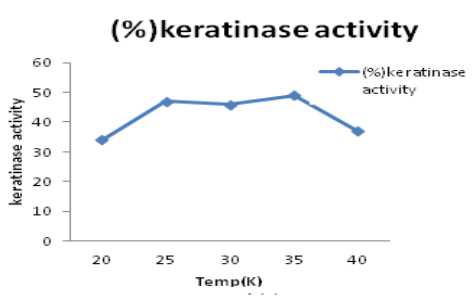

Figure.2.The above graph showed the maximum keratinase activity in the range of temperature between (25°C -35°C).

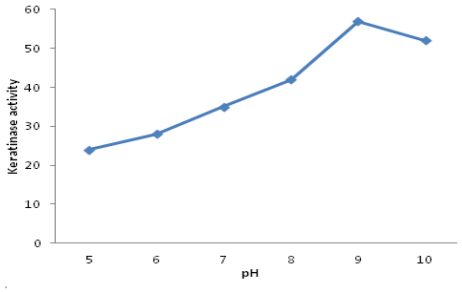

Figure.3.The graph plotted between the percentage keratinolytic activity and pH showed the maximum enzymatic activity between the ranges of pH (8-9)

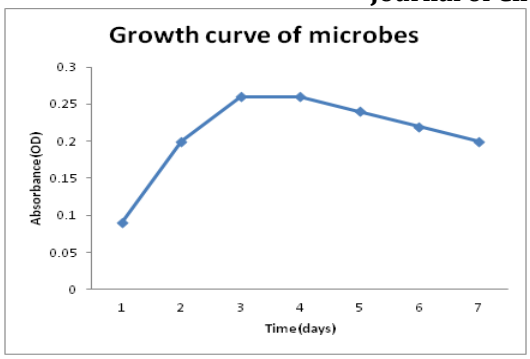

Figure.4.The microbes isolated from the soil showed maximum growth rate in the first three days. In the next two days growth rate reached at stationary phase and then declined growth rate

The consensus sequence of isolated microbes from the soil of poultry farm named as Nocardiopsis Alba DSM 43377.

CGGGGGTGACGTTGTCCGGATATTGGGCGTAAGAGCTCGTAGGCGGCGTG
TCGCGTCTGCTGTGAAAGACCGGGGCTTAACTCCGGTTCTGCAGTGGATA
CGGGCATGCTAGAGGTAGGTAGGGGAGACTGGAATTCCTGGTGTAGCGGT
GAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCTCTGG
GCCTTACCTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGA
TACCCTGGTAGTCCATGCCGTAAACGTTGGGCGCTAGGTGTGGGGACTTT
CCACGGTTTCCGCGCCGTAGCTAACGCATTAAGCGCCCCGCCTGGGGAGT
ACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCG
GCGGAGCATGTTGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGTT
TGACATCACCCGTGGACCTGTAGAGATACAGGGTCATTTAGTTGGTGGGT
GACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA
AGTCCCGCAACGAGCGCAACCCTTGTTCCATGTTGCCAGCACGTAATGGT
GGGGACTCATGGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACCA
CGTCACGTCATCATGTCCCTTATGTCTTGGGCTGCTAACATGCTACAATG
GCCTGTACAATGGGCGTGCGATACCGTAAGGTGGAACGAATCCCTTAAAG
CCGCCTCAGTTCTGATTGAGGACTCCAATTCCACGTAAGGAAGGGGGACC
CCATGTTTTACGCGGATGGTTGGTTTTTTTTAGCCGCCCTTCTTGCCCTC
ACGCCCTCAAGCCAAAAAACAACTGGATTTTCGGTGGGCGGAAAAGAGGG
TGACCCCTCGAGCGTACTTTCCCCCCCGGCGAAGTCCTTTACGAAGGTTT
CTCCCCTGGGGGAATTTCCCACGAGGCGGCCCCTTTAAAGTGTTGGGGGG
AAGTGTGGCTTCTGTGTTGGCCCGCTCCCCCCCCAAGAATGTTTCCTAAA
ATTCCCCTGGGAGAGGCGTCCCTCTCTCCCAAGTTTTCAAGCCCGTATGC
GGCGGCGCCAGAGGGAGGAAACACTTCGTCCGCCTCCATCCGAGAGAGGA
GGGGGGAATCACCGTGGAAAATAAGGGGCCCCTTTCCTCTTCTTAACGAC
AAAAAAGGGGGAGGAAGAGGGGGCCGTGTTGTTTACTTTCCCGCCGCCCT
TCCCCCCGGGGGGGCGGGACCCCAGCACACCCCCTCTTCATCACGTTCTC
TTCGTTTGTTTTGTTTTGTTTGGGCCGCCCCCCCCCTCCCTCGCTCCCC

Gene sequence of *Nocardiopsis alba*

From the Figure.1 the test tubes having Peacock and hen's feathers showed higher (OD) value as compared to other substrate. The (OD) value showed here indicates the amount of amino acids released by the keratinolytic activity of actinomycetes. As the enzyme is very sensitive towards temperature and pH, the keratinase enzyme produced by the microbes was stable in the range of temperature from 25°C to 35°C and the keratinolytic activity was very high in this range. So, the microbes obtained from the soil were mesophilic in nature.

Keratinolytic activity increased with increase in pH value which was in the range from 8 to 10 but the highest activity obtained on the value of pH 9. So the obtained microbes were alkalophilic in nature. The obtained strains could utilize feather as carbon and nitrogen source but the addition of yeast extract in low concentration was helpful for growth of actinomycetes. The growth in feather medium started intensively after lag period of 2-4

days and the pH of the culture filtrate moved towards the alkalinity with time. The pH value was higher in those which degraded the feather faster than the slow degrader. This was the characteristics of strong keratinolytic microbes. The keratinolytic actinomycetes attacked the feather enzymatically as well as mechanically. Mechanically colonization of feather resulted in the surface erosion and invasion into the keratinised structure weakens the feather.

CONCLUSION

We can conclude that keratinolytic activity of the enzymes produced by *Nocardiopsis alba* is very useful in the scavenging activity of poultry waste being generated in huge quantities day by day. The process is pollution free and leaves end product which degrades quickly in the soil. The subject gives a lot of scope for further research and there is a need to develop new strains which can generate proteolytic enzymes effectively.

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