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UGC letter No. File No: 47-506/12 (WRO) (date: 07-03-2013)

THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. Name of Principal Investigator: Dr. Shilpa Ashok Jani

2. Department: Microbiology, J and J College of Science, Nadiad-387001, Gujarat.

Name of College: J and J College of Science, college road, Nadiad-387001, Gujarat.

3. UGC approval Letter No. and Date: File No: 47-506/12(WRO) 7 march, 2013,

4. Date of Implementation: April-2013

5. Tenure of the project: Two years.

6. Total grant allocated: 1,75,000.00 Rs. (One lakh seventy five thousand rupees only).

7. Total grant received: 1,49,000.00 Rs. (One lakh forty nine thousand rupees only).

8. Final expenditure: 1,49,000.00 Rs. (One lakh thirty nine thousand rupees only).

9. Title of the Research Project: "Screening of keratinolytic actinomycetes and study of their enzymatic potential"

10. Objectives of the project:

1. Screening of efficient keratinolytic actinomycetes from various sources.
2. Measure of keratinolytic protease activity by various isolates.
3. Study of possible applications of this activity of actinomycetes.

11. Weather the objectives were achieved:

The project entitled **Screening of keratinolytic actinomycetes and study of their enzymatic potential" has** achieved all the objectives during its tenure. Identification of the potent protease producing actinomycete. Identification process is done that of few actinomycete cultures potent for feather degradation with 16 S r RNA sequencing at Gujarat Biotechnology Mission , Gandhinagar. Checking of feather degrading capacity of isolates and protease produced by the isolates was carried out and All the isolates are employed to check their feather degrading capacity out of which, many shown extraordinary results. These results may be much useful for applied aspects of feather waste management and production of amino acids from feather waste.

12. Achievement from the project:

Present study has achieved a tremendous applicability in remediation of feather waste and synthesizing amino acids from this robust waste. Keratin-containing materials are abundant in, nature but have limited uses in practice since they are insoluble and resistant to degradation by the common proteolytic enzymes. 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. Keratinolytic enzymes that may have potential roles in biotechnological processes involving keratin containing wastes from poultry and leather industries. The potential use of keratinase is in different application where keratins should be hydrolysed such as the leather and detergent industries, textiles waste bioconversion, medicine and cosmetics for drug delivery through nails and degradation of keratinized skin, besides it involves in the hydrolysis of prion proteins that arise as novel outstanding applications of the enzyme. Actinomycetes genera are becoming increasingly important as a source of novel products. So in this study we characterized many keratinolytic protease from S*treptomyces sp.and Bacillus spp* .We can understand all these points referring to our papers pblished in various journals as listed below.

I am much happy that many students of post graduate department of Gujarat University have registered for dissertation under this project and successfully completed their work.

Their poster presentations and oral presentations listed here are proof of how this project benefited these post graduate students.

13. Summary of the Findings:

Keratins are insoluble proteins from feathers, wool, silk, collagens, elastin, horn, hair and nail. They are not easily degraded by common proteolytic enzymes like trypsin, pepsin and papain.The resistant property of these compounds are due to their disulphide bonds, hydrogenbonds, salt linkages and cross linkages and hydrophobic interactions. Actinomycetes are known to digest keratins by synthesizing specifi class of extracellular enzymes called alkaline thermo stable proteases which degrade keratin into small peptides that can be utilized by cell.During this project work, many Alkaline protease producing thermophilic actinomycete strains were screened from various locations where there is possibility of presence of actinomyces like Dalhousie (H.P.)rock sample, Sheriyaj(Junagadh, Gujarat) poultry waste, Mehmdavad (Gujarat) road poultry waste, Fatepura (Gujarat) hair debris Mehrav (Gujarat) poultry waste,hot water spring of Tulsishyam Gujarat.

Many from them were identified on the basis of colony characters, biochemical activity, spore nature, growth patterns and pigmentation and 16 S r RNA sequencing.The partially purifi ed protease of many the isolates were employed to check feather degradation. The feathers were degraded successfully within 72h at 45ºC. The degraded samples were analyzed for release of various amino acids by HPLC- Fluorescence with post column Derivatization. And the dissertation students yried to identify the amino acids by performing paper chromatography of the digested samples.The aminoacids released were tyrosine, phenylalanine, leucine, valine, cysteine, arginine, methionine, etc. And most of the isolates were found to have significant keratinolytic activity and may serve dual purpose for degradation of poultry waste and production of amino acid rich feed supplement. The protein rich, concentrated feather meal can also be used for organic farming as semi-slow release, nitrogen fertilizer.

The main isolates identified are

Saccharothrix xinjiangensi, **Strain B-4** ***Bacillus flexus , Saccharomonospora viridis*** **Sj-21,** **S***treptomyces spp etc.*

14. Contribution to the society:

Proteases possess some characteristics of biotechnological interest due to which they have become the most important industrial enzymes .Almost all proteases are heat resistant , vary widely in their specific activities, optimum pH, pH stability range (2-13), heat sensitivity, substrate specificity, active site, catalytic mechanism and stability profiles . The proteases that have pH optima in the range of 8.0-11.0 are grouped under the category of alkaline proteases. Some of the important alkaline proteases are solanain, hurain and proteolytic enzymes of *Bacillus* and *Streptomyces* species. Alkaline proteases are robust enzymes with considerable industrial potential in detergents, leather processing, silver recovery, medical purposes, food processing, feeds, and chemical industries, as well as waste treatment. These enzymes contribute to the development of high value-added applications or products by using enzyme-aided (partial) digestion.

The protease of *Actinomycetes* which we have studied for are with broad range of substrate specificity so can be recommended for recovery of silver from photographic films (Gelatin hydrolysis), for animal feed industry and clearing beverages, in leather industry for dehairing and bating skins (Keratin hydrolysis), production of amino acid and peptides from poultry waste (Keratin hydrolysis) and can be recommended as detergent additive (Thermostable, serine, alkaline and detergent compatible).

In the above light, with the advent of latest technology the potential of these modern efficient enzymes will be much greater with many fold increased applications. Thus, in this century with so many challenges to face enzyme technology may offer a great advantage in day to day life. In recent year broad specificity of the hydrolytic action of proteases find most robust enzyme in Biotechnological applications as well as structural elucidation of proteins. This study was fruitful in identifying such enzyme, which can be exploited commercially. And hence, in service of Society too.

15. Weather any Ph.D. enrolled/produced out of the project: YES,

17 seventeen students of post graduate department of Microbiology and biotechnology of Gujarat University are benefited to get their dissertation work under this minor research project and got chance to present their posters at various symposia.

16. Number of publications out of the project:

A. Research Paper:

1. Study of Keratinolytic Activity of Thermophilic and Alkaliphilic Actinomycetes: *Saccharomonospora Viridis* SJ-21 479 *Shilpa Jani and Harshad Patel* International Journal of Agriculture, Environment & Biotechnology Vol. 7, Special Issue, 409-510: July 2014

2. Screening, isolation and characterization of keratin degrading actinomycetes: *Streptomyces* sp. and *Saccharothrix xinjiangensi* and analyzing their significance for production of keratinolytic protease and feed grade
Aminoacids Shilpa Ashok Jani1*, Rishit Soni2, Hetal Patel2, Brinda Prajapati2 and Gayatri Patel2

3. Production and characterization of keratinolytic protease
from *Streptomyces* sp Shilpa Ashok Jani1*, Raval Heta2, Harnisha Patel2, Drashti Darji2, Ankit Rathod2 and Seema Pal2 *Int.J.Curr.Microbiol.App.Sci* (2014) 3(9) 940-955
Int.J.Curr.Microbiol.App.Sci (2015) 4(5): 962-975

4. Screening and Characterization of Alkaline Protease Producing Bacillus Strain B-4 *Bacillus flexus* and Study of its Potential for Alkaline Protease Production Shilpa A. Jani, 1*, Yesha M. Parekh2, Tanvi N. Parmar2, Tulsi J. Dalwadi2, Hetal B. Patel1 and Sagar K. Parmar2 *International Journal of Current Microbiology and Applied ciences ISSN: 2319-7706* Volume 5 Number 5 (2016) pp. 767-787 Journal homepage: http://www.ijcmas.com

5. Production of Alkaline Keratinolytic Protease by *Bacillus* sp. B13 from Feather Waste Shilpa Ashok Jani1*, Sumaiya Malek2, Anvi Patel3, Krupa Pathak2 and Kinjal Baria2 *International Journal of Current Microbiology and Applied Sciences*
ISSN: 2319-7706 Volume 6 Number 5 (2017) pp. xx-xx
Journal homepage: http://www.ijcmas.com.

B. **Oral presentations:**

1. STUDY OF KERATINOLYTIC ACTIVITY OF THERMOPHILIC AND ALKALIPHILIC ACTINOMYCETES: *SACCHAROMONOSPORA VIRIDIS* SJ-21 at, Department of Microbiology and Department of Biotechnology, Genetics & Bioinformatics On Wednesday February 26, 2014, N.V. Patel institute of pure and applied Sciences,Vallabh vidyanagar.

2. CHARACTERIZATION OF ALKALINE PROTEASE OF ACTINOMYCETES *SACCHAROMONOSPORA VIRIDIS* SJ-21 By Dr. Shilpa Ashok Jani Associate Professor & Head, Microbiology Department, J and J College of Science , Nadiad, at School of SciencesAhmedabad, Gujarat, India. Date: 15/03/2019 at

C. **Poster presentations:**

1. KERATIN DEGRADATION BY ENZYMES OF *STREPTOMYCES SP*. (A1) AND *SACCHROTHRIX XINJIANGENSIS* (A2) AND THEIR SIGNIFICANCE FOR AMINO ACID PRODUCTION FROM FEATHERS

SHILPA ASHOK JANI[1], RISHIT SONI AND HETAL PATEL Presented at UGC Sponsored National Conference on "Spectroscopy and Stereochemistry"19th December 2015(Saturday), J and J college of Science, Nadiad-387001,Gujarat,India

2. SCREENING AND CHARACTERIZATION OF KERATIN DEGRADING ACTINOMYCETES: *SACCHAROTHRIX XINJIANGENSIS* AND ANALYZING ITS SIGNIFICANCE FOR PRODUCTION OF KERATINOLYTIC PROTEASE AND FEED GRADE AMINOACIDS Shilpa Ashok Jani[1]*, Rishit Soni[2], Hetal Patel[2], Brinda Prajapati[2] and Gayatri Patel[2] Presented at UGC Sponsored National Conference on "LATEST DEVELOPMENTS IN BASIC APPLIED SCIENCES."10th Jan, 2015(Saturday), M.B.PATEL SCIENCE COLLEGE, Anand-388001,Gujarat,India

3. ISOLATION AND CHARACTERIZATION OF KERATINOLYTIC ALKALINE METALLO PROTEASE FROM ACTINOMYCETES: *STREPTOMYCES SPP*
Hetal B. Patel, Shilpa Ashok Jani and Rishit A. Soni Microbiology Department, J and J college of Science, Nadiad, Gujarat, India. Presented At Xxx Gujarat Science Congress-2016 "Challenges For Science And Technology Education During Coming Decades: Preparing For A Sustainable Gujarat" 6 And 7 February,2016, K.S.K.V Kachchh University, Bhuj.

4. COMPARATIVE STUDY OF ALKALINE KERATINOLYTIC PROTEASE PRODUCTION ABILITY OF ACTINOMYCETES: *STREPTOMYCES SPP, SACCHAROTHIX XINJIANGESIS, SACCHAROMONOSPORA VIRIDIS AND THERMOACTINOMYCETES VULGARIS* Hetal B. Patel, Shilpa Ashok Jani and Rishit A. Soni Microbiology Department, J and J college of Science, Nadiad, Gujarat, India. PRESENTED AT NATIONAL CONFERENCE ON "MICROBIAL BIOTECHNOLOGY:CLUSTERING RESEARCH AND INDUSTRIAL DEMAND." 20 AND 21 FEB,2016 AT DEPARTMENT OF MICROBIOLOGY AND BIOTECHNOLOGY,GUJARAT UNIVERSITY,AHEMDABAD.