Biodegradation of Chicken Feather Waste Using Keratinase Producing Microbes

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FINAL REPORT

Submitted By

Dr. C. RAVI
Assistant Professor
&
Principal Investigator
Department of Zoology
Thiagarajar College
(Re-accredited with 'A' grade by NAAC)
Madurai – 625009

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University Grants Commission
Bahadurshah Zafar Marg
New Delhi – 110002

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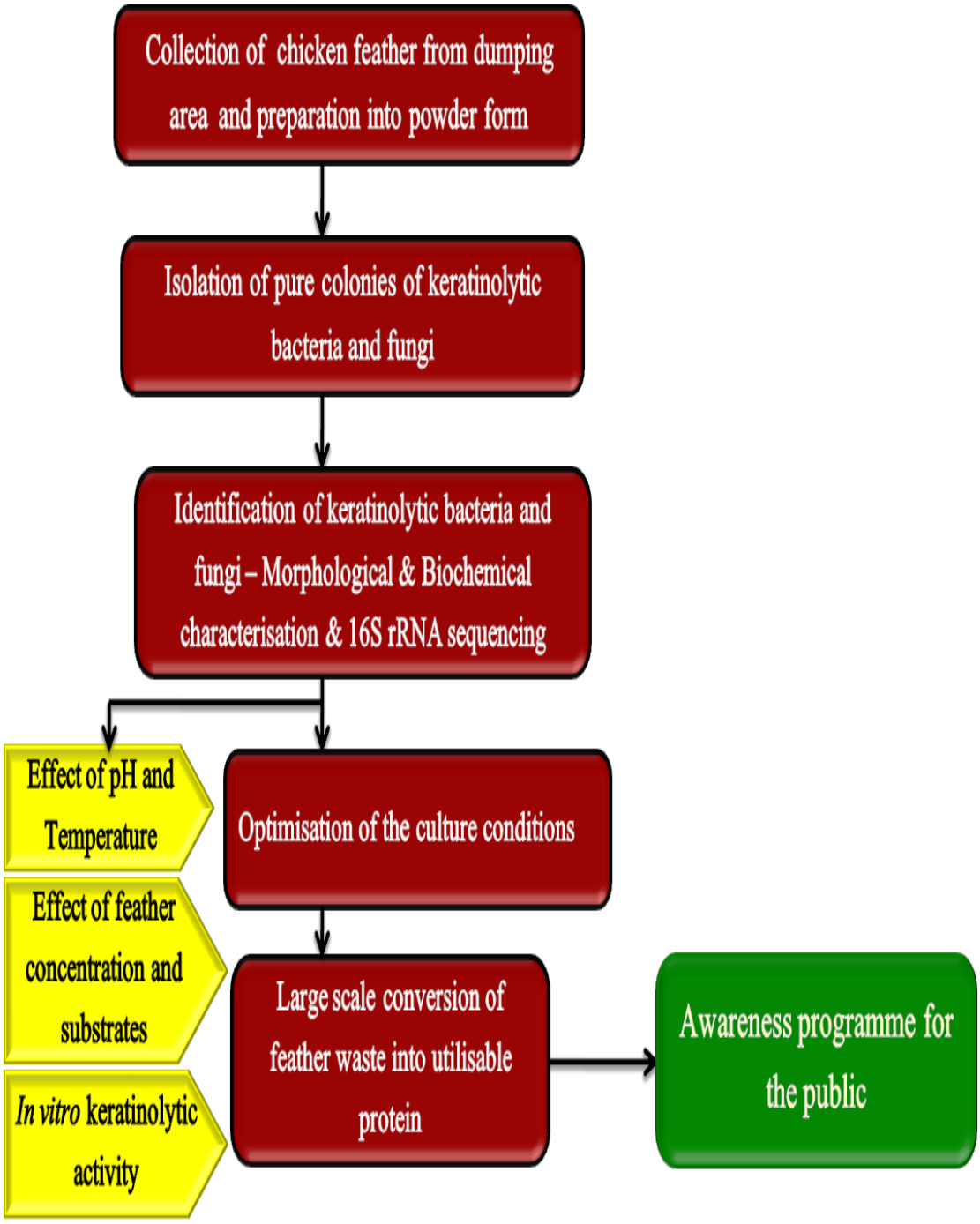
OBJECTIVES

1. Isolation and screening of the feather degrading bacteria and fungi and selection of highly effective bacterial and fungal strains.
2. Identification of the bacterial and fungal strains by biochemical characterization and 16s rRNA sequencing for strain confirmation.
3. Temperature, pH and substrate optimization for the growth of the isolated feather degrading bacteria and fungi.
4. Mass production of keratinase and determination of keratinolytic activity of the isolated enzyme.
5. Large scale degradation of the feather waste using the potential bacterial and fungal strains in fermenter and the recovery of proteins.
6. To create awareness to the public on the effect of chicken feather accumulation and its biodegradation.

Fig. 1. Schematic representation of work plan



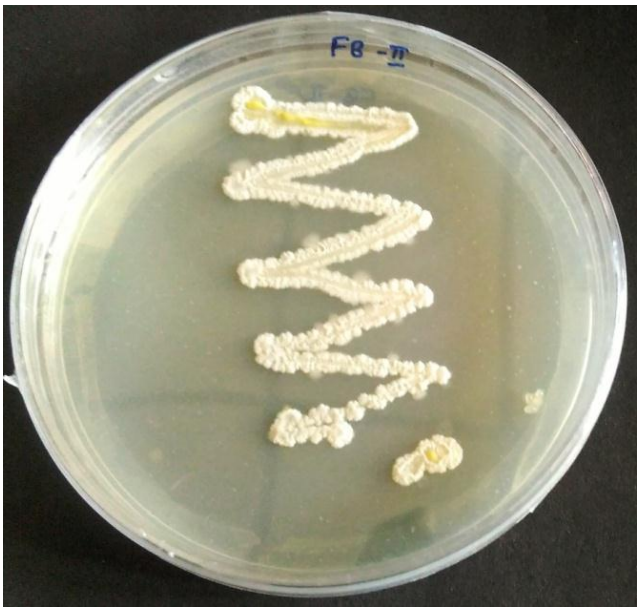
SUMMARY

A study was undertaken to isolate bacteria and fungi capable of degrading keratin present in the chicken feather waste. Soil samples were collected from feather dumping sites of Teppakulam (Madurai) and Virudhunagar. Four different bacteria and six different fungi were isolated from Teppakulam soil and fourteen bacteria and six fungi were isolated from Virudhunagar soil. Temperature, pH, substrate and concentrations of feather were optimized for the effective growth of the microbes and growth of bacteria and fungi were assessed based on optical density method. Keratinolytic activity was determined by estimating total protein in the cell free media and keratinase assay. The bacteria were identified based on morphological and biochemical tests and fungi were identified by morphological and microscopic examination. Three better bacterial strains were further sequenced by 16S rRNA sequencing and confirmed to be *Bacillus licheniformis, Arthrobacter arilaitensis* and S*erratia marcescens* respectively. Three fungi such as *Aspergillus niger, Aspergillus flavus and Aspergillus terrus* were confirmed by ITS primer sequencing method. The sequences of bacteria and fungi are deposited in NCBI. Among the isolates, one of the best bacteria, *Serratia marcescens* (I) from Teppakulam soil and *Arthrobacter arilaitensis* from Virudhunagar soil and fungi, *Aspergillus niger* (from both the soil) were chosen based on their growth, protein concentration and keratinase activity respectively. The optimum temperature, pH, substrate and feather concentration for the growth of *S. marcescens* (I) was 58 ^{o}C, pH 9.5, Casein and 1g feather respectively. For *S. marcescens* (II), it was 45 ^{o}C with pH 9.5 and 1g feather and for *Aspergillus niger* (I) it was 32 ^{o}C and pH 9, BSA and 1g feather respectively. The optimum parameters for the growth of *Arthrobacter arilaitensis* were 40 ^{o}C, pH 8.5 and 1g feather and for *Aspergillus niger* (II) it was 25 ^{o}C with the pH9 and 1.5g feather respectively.

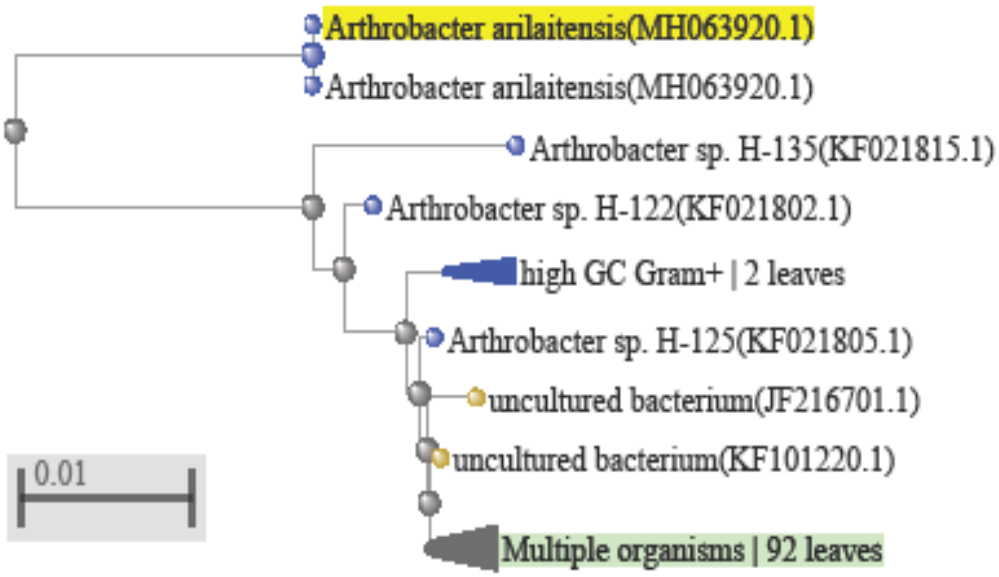
Fermentation process was carried out for bacteria isolated from Virudhunagar soil namely *Bacillus licheniformis*, *Arthrobacter arilaitensis* and *Serratia marcescens*. The amino acid and protein content of the hydrolysates were estimated for every 24 hrs. Further, smear of the feather from the fermentation tank was prepared for every 24 hours and observed. In the fermentation process, *A. arilaitensis* produced more protein (2.14 mg/ml) and amino acid (0.535µg/ml) that was the testimony of its better degradation ability. The feather smear observations also confirmed the same. Antibiotic sensitivity assay revealed that the isolated bacteria *A. arilaitensis* was sensitive to most of the antibiotics, however, it was resistant to Ampicillin, Penicillin and Amoxicillin which is a bit of concern. Based on the obtained results, it has been concluded that *A. arilaitensis* has a greater ability to degrade feather when optimum conditions are provided and it could be employed in the chicken feather waste management programme.

RECOMMENDATIONS BASED ON PRESENT STUDY

1. The keratinase producing bacteria *Arthrobacter arilaitensis* could be used in the degradation of chicken feather waste under controlled laboratory conditions.
2. *A. arilaitensis* is sensitive to the commonly used antibiotics such as Tetracyclin, Gentamycin, Streptomycin, Norfloxacin, Erythromycin, however it is resistant to Ampicillin, Amoxyilin and Penicillin. Hence precautionary measures must be taken before inducting this strain for mass feather waste management programme.
3. If awareness created to general public properly on the harmful effects of unscientific disposal of chicken feather waste, fruitful results could be achieved as witnessed in the present study.



a. *Arthrobacter arilaitensis*



b. Phylogenetic tree within the genus *Arthrobacter* sp.

Plate 1: The better feather degrading bacterial strain (*Arthrobacter arilaitensis*) isolated in the present study and the phylogenetic tree based on 16S rRNA sequence of the isolated bacterium within the genus *Arthrobacter* sp.

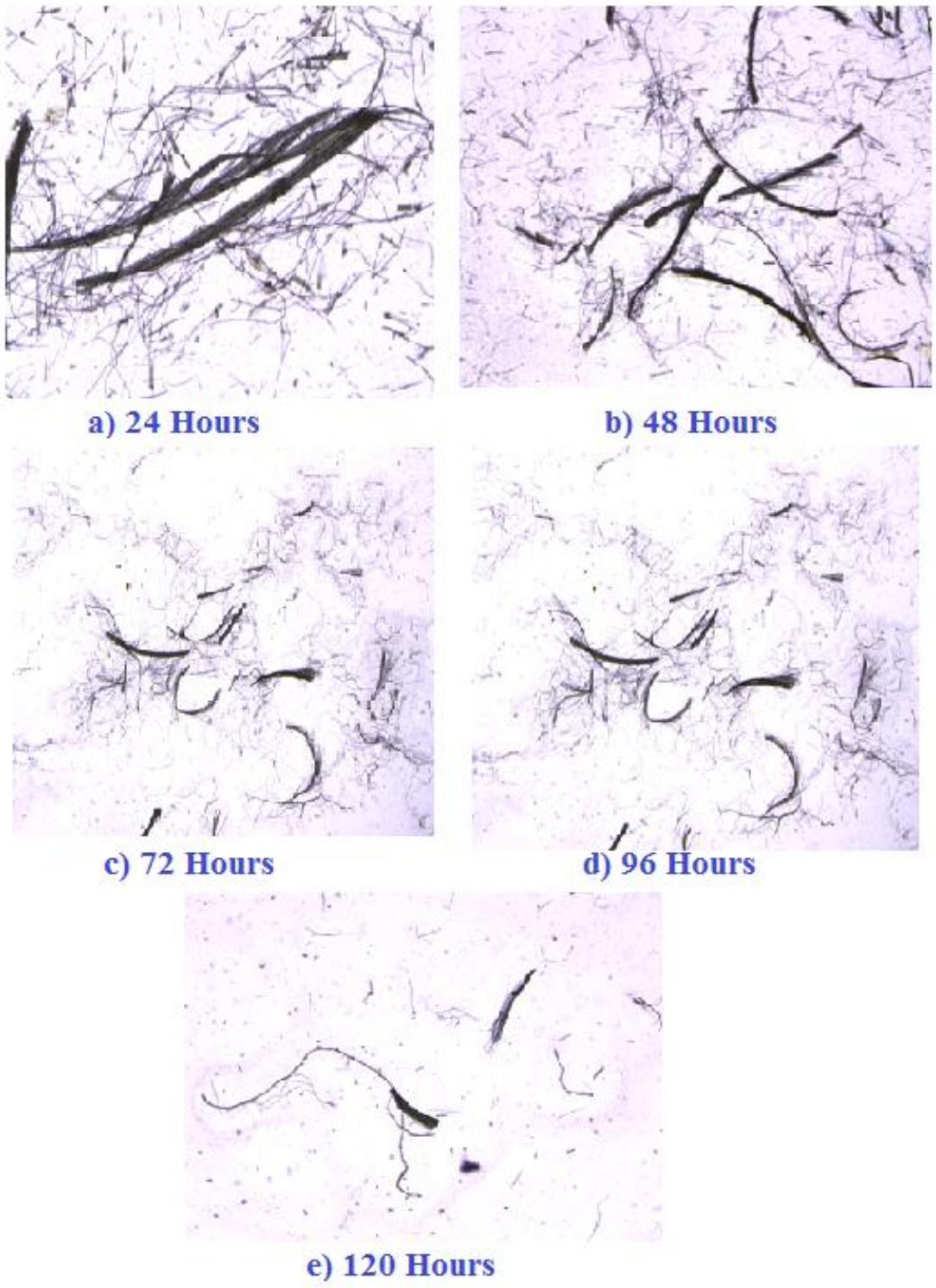


Plate 2: Microscopic view of the feather degraded during fermentation by *Arthrobacter arilaitensis*

a. Virudhunagar

b. Kariapatti

c. Madurai

d. Feather dumped in to the river Vaigai

e. Dindigul

d. Dindigul (road side)

Plate 3: Indiscriminate dumping of feathers on to the environment


Plate 4a: Creating awareness to the people for the proper disposal of feather wastes


Plate 4b: Cleaning the feather dumping sites by the people after the awareness programme