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RESEARCH ARTICLE

ISOLATION IDENTIFICATION AND CHARACTERIZATION OF FEATHER DEGRADABLE BACTERIA IN THANJAVUR (DT)

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

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

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ABSTRACT

Feather waste are generated in large quantities as a byproduct of commercial poultry processing as well as through natural process. Feathers are made up of primarily keratin which is resistant to common proteolytic enzymes such as pepsin, trypsin and papain. Environmental pollution and degradation of ecosystem have assumed significance owing to an increase in the accumulation of the wastes from industries, agriculture and poultry. In India, poultry feathers, animal hairs and other keratin sources are being thrown. Keeping this in mind, the present study was planned to isolate and identify the effective feather degrading bacterial organisms. The soil sample from Thanjavur Station was taken for isolation of bacterial colonies using Nutrient Agar Medium. The bacterial organisms were identified and the effective strain was used for further study.

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INTRODUCTION

Feather waste, generated in large quantities as a by product of commercial poultry processing, is nearly pure keratin protein. Keratin in its native state is not degradable by common proteolytic enzymes such as trypsin, pepsin, and papain.(Williams *et al*., 1990). Worldwide around 25 billion chickens are killed annually. The annual production of poultry meat is about 50,000 tonnes, with an annual increase of about 4.5%. Feathers represent 5-7% of the total weight of mature chickens. Feather is pure keratin protein and is insoluble and hard to degrade due to highly rigid structure rendered by extensive disulphide bond and cross – linkages. The keratin chain is insoluble, high stable structure tightly packed in the "α-helix ("α-keratin) and β-sheets (β-karatin) into super coiled polypeptide chain (Parry and North, 1998). Feathers are also a good source of soil organic matter which plays a key role in maintaining soil fertility and productivity. The effect of the organic matter may be either direct or indirect. Organic matter acts as a direct source of plant nutrients and in an indirect manner influence the physical and biological properties of the soil (Chesworth, 2008). Feathers are the major waste by-product of chicken poultry and it is gradually increasing day by day. Specifically, around 8.5 billion tons of chicken feathers are produced annually world as a waste from the production unit of chicken meat and the Indias contribution alone is about 350 million tons (Tanmay paul *et al*., 2013).

Keratin is an insoluble protein macromolecule with very high stability and low degradation rate. Keratin is mainly present in hair, feather, nails, wool and horns. High protein content of keratin waste can be used as a good source of protein and amino acids by systematic recycling. Keratin is an extracellular enzyme used for the bio degradation of keratin. Kearatinase attacks the disulfide bond of keratin to degrade it (Prasad *et al*., 2011).

MATERIALS AND METHODS

Collection of soil sample

Feather dumping soils were collected from Orathanadu (TK) Thanjavur (DT), Tamilnadu, India during May 2014. Soil samples were collected from 3 to 4 cm depth and transferred in sterile plastic bags. The samples were brought to Microbiology Research lab for further processing.

Corresponding author: Tamilkani, P.
Department of Microbiology, A.V.V.M Sri Pushpam College (Autonomous), Poondi – 613503, Thanjavur (Dt), Tamil Nadu, India.

Analysis of physico-chemical parameters of the soil (Ibrahim 2014)

Physical parameters

To investigate physical parameters such as soil Texture, pH, Bulk density, Water holding capacity, Electrical conductivity and Oraganic carbon.

Chemical parameters

To investigate chemical parameters include salinity, Macro nutrients (Nitrogen, Phosphorus & Pottasium). Available Micro nutrients (Zinc, Copper, Ferrus, Magnesium and Boron).

Preparation of soil suspension

1 gm of feather dumped soil sample transferred in 9 ml of sterile distilled water. The samples were serially diluted upto 10^{-9} and 10^{-4} to 10^{-5} was fold dilution was plated.

Isolation of feather degrading bacteria

Nutrient agar medium was prepared and sterilized at 121°C temperature for 15 minutes in autoclave. Medium was poured on sterile petridishes once its reached the tolerable temperature (45°C) and allowed to solidify. Spread plate techinique was followed to isolate the bacteria. Each plate was received 0.2 ml of 10^{-4}, 10^{-5} dilution of the inoculum. The plates were incubated at room temperature and examined the plates 18-24 hours. After incubation the colonies with different morphology were picked and purified using streak plate method.

Characterization and identification of feather degrading bacteria (Prasad *et al.*, 2010) cultural characterization

The bacterial isolates were observed under the microscope, The colony morphology was recorded with respect to colour, shape, size, nature of colony pigmentation.

Microscopic observation

The bacterial isolates were Gram stained and observed under a high power magnifying lens in light microscope. Endospore staining and motility test were performed to observe the morphology and motility of the cells.

Biochemical characterization

The bacterial isolates were characterized biochemically by Indole, Methyl Red, Voges Proskauer, Citrate utilization, Triple sugar iron, Catalase, Nitrate, Urease, Starch hydrolysis, Gelatin hydrolysis, Lipid hydrolysis, Casein hydrolysis, Carbohydrate fermentation, Salt tolerance and Oxidative fermentation test (Glucose, Lactose and Sucrose).

RESULTS AND DISCUSSION

Keratin is a strong protein find in skin, hair, nails, horns, teeth. Keratin is difficult to dissolve due to the presence of cysteine disulfide that can form disulfide bridges. The disulfide bridges create an extremely strong helix shape. The feather degrading bacterium isolated from soil was identified by studying its microscopic, biochemical and cultural characteristics.

Physico-chemical parameters of the soil

Ibrahim *et al.* (2014) stated that analysis of the soil chemical profile showed that amended soil had the highest values for soil pH(7.12±0.02), organic carbon (1.10±0.10), nitrogen (0.09±0.01), phosphorus (0.53±0.02 mg/kg) and magnesium with (0.45±0.01 mg/kg) these values decreased along the time course of the experiment with Nitrogen returning to the same value as the control (0.05±0.20%) after three weeks. In the present study, the physical parameters of the soil sample showed that the soil Texture (resembles) was clay loamy, pH of about 6.2, bulk density showed 0.945 g/cm^3, water holding capacity of the soil was 27%, electrical conductivity was 0.640(dsm^{-1}) and organic carbon was about 0.69% (Table 1). The chemical parameters of the soil showed the Salinity of 1.40, For macronutrients analysis, nitrogen content was about 11.0%, phosphorus 8.50%, potassium 4.2%, for micronutrient analysis Zinc of about 0.90%, copper 1.45%, ferrous 1.80%, manganese 1.65%, boron 0.920% was recorded is the present investigation (Table 2).

Characterization and identification

The identification of keratinolytic bacteria was based on cell morphology, colony morphology, and several biochemical test. SN1, SN2, SN3 were determined to be Gram-positive, sporulating, motile bacilli. The isolate SN1, SN2 formed yellow colored colonies and SN3 showed white colored colony on feather meal agar plate. These results suggested that these three strains belong to genus bacillus. On the basis of morphological characteristic and cultural characteristic on Hicrome *Bacillus* agar and was identified. They (SN1, SN2 and SN3) were further identified to be as sample *B.megaterium, B.thuringenesis, B.pumilis* respectively. These strains degraded the chicken feathers and pigeon feathers completely. Their cultural characteristic in the differential media with *B.megaterium* SN1 showed yellowish green, irregular colonies *B.thuringenesis* SN2 showed blue circular colonies, *B.pumilis* SN3 showed green, flat, circular shiny colonies (Sarita Agrahari and Neeraj Wadhwa, 2010). In the present study, the microscopic observation showed that all the bacterial samples were Gram positive. Maximum number of isolates were cocci in shape, The colony characteristic showed rapid growth, mostly circular in shape. Ibrahim *et al.* (2014) stated that analysis of the viable bacteria population showed that the amended and control soil are *Bacillus* sp., *Proteus* sp., *Staphylococcus* sp and *Actinomyces* sp. *Actinomyces* sp was identified after three weeks amendment with the fermented feather and the other group of bacteria appears to be stable members of the soil microbial community. *Bacillus* sp are dominant group of organisms with 58.8% while *Proteus* sp and *Actinomyces* sp were the least the encountered bacteria group with 5.9% occurrences each. In the Biochemical tests the bacterial samples showed the positive results in Indole test. Maximum number of isolates were showed negative results in Methyl Red test and Voges Proskauer test. All isolates were Gram positive, rod shaped and spore former and were able to utilize both glucose and sucrose but not lactose.

Table 1. Analysis Physical Parameters of soil

Sampling Place	Soil Texture	pH	Bulk density (g/cm^3)	Waterholding capacity(%)	EC (dsm^{-1})	Organic Carbon(%)
Orathanadu	Clay loamy	6.2	0.945	27	0.640	0.69

Table 2. Analysis Chemical Parameters of soil

Sampling Places	Salinity (PPT)	Macro Nutrients(%)			Micro Nutrients(%)				
		N	P	K	Zn	Cu	Fe	Mn	B
Orathanadu	1.40	11.0	8.50	4.2	0.90	1.45	1.80	1.65	0.920

Table 3. Morphological, Physiological, Biochemical characteristic of ten isolated bacterial strain

Details of Experiment	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
Gram character	+	+	+	+	+	+	+	+	+	+
Shape of bacteria	Rod	Cocci	Cocci	Cocci	Cocci	Rod	Rod	Cocci	Rod	Rod
Growth	Rapid	Rapid	Rapid	Rapid	Rapid	Rapid	Rapid	Rapid	Rapid	Rapid
Shape	Circular	Circular	Irregular	Circular	Irregular	Irregular	Circular	Circular	Circular	Irregular
Indole	+	+	+	+	+	+	+	+	+	+
Methyl Red	+	-	-	-	-	-	-	+	-	-
Voges Proskauer	-	-	-	-	-	+	-	-	-	-
Citrate utilization	+	+	+	-	+	+	+	-	+	+
Triple sugar Iron	H_2S production	Acid slant	Acid slant	Acid slant	Acid slant	H_2S production	H_2S production	Acid slant	Acid slant	H_2S production
Catalase	+	+	+	+	+	+	-	+	-	+
Nitrate	+	+	+	+	+	+	-	+	+	-
Urease	+	-	-	-	-	-	-	-	-	+
Starch Hydrolysis	+	-	+	+	-	+	-	+	-	+
Gelatin Hydrolysis	+	+	+	+	-	+	-	+	+	+
Lipid Hydrolysis	+	+	+	+	+	+	+	+	+	+
Casein Hydrolysis	-	+	+	+	-	+	-	+	+	-
Carbohydrate fermentation	+	+	+	+	-	+	-	+	+	+
Salt Tolerence	-	+	+	+	+	+	+	-	+	+
Glucose	+	-	+	+	-	+	-	-	-	-
Lactose	-	-	-	-	-	-	-	+	-	+
Sucrose	+	+	+	+	-	-	-	-	+	+

(+) – Positive, (-) – Negative

Table 4. Identification of Bacterial strain

S.No.	Strain	Bacterial Isolates
1.	B1	*Bacillus cereus*
2.	B2	*Staphylococcus aureus*
3.	B3	*Microbacterium arborescens*
4.	B4	*Campylobacterium jejuni*
5.	B5	*Listeria grayi*
6.	B6	*Corynebacterium striatum*
7.	B7	*Clostridium butyricum*
8.	B8	*Micrococcus luteus*
9.	B9	*Enterococcus faecium*
10.	B10	*Brachybacterium faecium*

They were also catalase and oxidase positive. All isolates showed typical characteristics of *Bacillus* sp. Reports of several workers showed that the *Bacillus* sp was considered as prime producer of keratinase (Lin *et al*., 1995). Keratinolytic bacteria particularly from the genus *Bacillus* have been isolated from the plumage and bird feathers (Burtt and Lchida.,1999 Lchida *et al*., 2001). In lipid hydrolysis, the results were positive for all the samples, in Oxidative fermentation test the maximum samples take the sugar Glucose and Sucrose and not the Lactose. In Nitrate test, the maximum number of samples showed positive results. The maximum number of isolates showed negative results in urease test (Table 3).

Identification of Bacterial strain

The bacteria identified from these amended and control soil is mostly *Bacillus* sp including *Bacillus licheniformis, Bacillus brevis* and *Bacillus subtilis*, having 58.8% of occurrence. *Staphylococcus* sp include *S.chromogenes* and *S.epidermis*, has 29.4% while *Actinomycetes* sp and *Proteus mirabilis* had 5.9% each. (Kim *et al*., 2013; Jayalakshmi *et al.,* 2011; Lakshmi *et al*., 2013; VenkataNagaRaju and Divakar; 2013).

A bacterium isolated from poultry waste has been showed to degrade feather keratin by using feathers as a primary source of energy, carbon, nitrogen and sulfur. (Williams *et al*., 1990).

Totally ten Bacterial strains were identified as potential organisms for degrading the chicken feather from soil, as showed followed by *Bacillus cereus, Staphylococcus aureus, Microbacterium arborescens, Campylobacterium jejuni, Listeria grayi, Corynebacterium striatum, Clostridium butyricum, Micrococcus luteus, Enterococcus faecium and Brachybacterium faecium* (Table 4). Feather degrading bacterium were sucessfully isolated and identified. The bacteria and their keratinase could be applied for feather degradation for feed and fertilizer industries, wool cleaning for detergent industry. The useful of this enzyme preparation and its pure form could be exploited for waste treatment and also as animal feed supplement.

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