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Isolation of Keratinolytic Bacteria from Soil for the Bioconversion of the Poultry Feather Waste

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The present investigation dealt with the isolation of keratinolytic bacteria from soil and their utilization, for the bioconversion of the poultry waste feathers. The isolation was performed by serial dilution and spread plate method. The skimmed milk agar medium was used for the screening of bacterial strains for keratinolytic activity at 37°C for 24 h of incubation. These isolated organisms were incubated with production medium in shaking incubator at 40°C for 96h; the keratinase production was recorded in a range of 1.63 - 11.67 U/ml. The maximum keratinase production and bioconversion of feather waste was observed (80%) by bacterial isolate i.e., S3 (*B. licheniformis*) out of the fourteen bacterial isolates. These findings revealed the potential use of microorganisms for waste management as well as for industrial applications.

Key words: Keratinolytic bacteria, bioconversion, *B. licheniformis*, keratinase.

The biological solid waste let out by the by-product industries is a matter of concern for all of us. Keratinous waste like horns, feather, nails, hoofs, scales, and wools are increasingly accumulating in the environment generated from poultry and meat processing plants, slaughterhouses, tanneries, and other industries. Keratin protein present in keratinous waste does not degrade easily by commonly known proteases like trypsin, pepsin, and papain due to presence of disulfide bonds[2]. The chemical processes can convert these keratinous wastes into useful materials, but again, chemical processing cause's environmental pollution. To overcome these situations, microbial treatments are being considered with varying degree of success. The feather can be hydrolysed by keratinase which is a proteolytic enzyme specific to keratins. This enzyme has been produced by fungi, including the species of *Penicillium*[12] *Aspergillus*, *Onygena*, *Absidia* and *Rhizomucor*[6], *Aspergillus niger, Cladosporium cladosporioides, Metarrhizium anisopliae, Neurospora tetrasperma* and *Westerdikella dispersa*[7], a few actinomycete such as *Streptomyces pactum, S. albus, S. fradiae* and *S. thermoviolaceus*[3, 5, 14, 15] and Keratinases from bacteria are isolated and characterized. For instance, *B. subtilis* 1271, *B. licheniformis* 1269 and *B. cereus* 1268[11], *Bacillus subtilis*[4], *Bacillus halodurans*[17], *Pseudomonas aeruginosa*[20], *Bacillus weihenstephanensis*[19], *Bacillus subtilis* and *B. licheniformis*[10]. Biodegradation by these organisms offer an improved method for utilization of these waste materials into useful products. In this paper we successfully isolated keratinase producing bacteria from soil.

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MATERIALS AND METHODS

Sample collection

The soil samples were collected from different waste dumping areas of Allahabad, India. Soil samples were collected from 3 to 4 cm depth and transferred in sterile plastic bags.

Isolation of bacteria

Isolation of bacteria was performed by

serial dilution and plating method on nutrient agar medium (NAM). One gram of soil samples were transferred in 10 ml of sterilized distilled water and mixed properly. Serial dilution was done up to 10^{-6}. 0.1 ml of the diluted soil samples were inoculated in the NAM plates from each dilution. Plates were incubated at 37ºC for 24 to 48 hours. The bacterial isolates were further sub cultured on NAM to obtain pure culture. Pure isolates were maintained in NAM slants at 4ºC for further studies.

Screening of keratinolytic bacteria

Skim milk agar was prepared and the above colonies were streaked on milk agar plates for testing the caseinolytic activity of the organism. Isolates were inoculated onto plates and incubated at 37°C for 24 h. Strains producing clear zones in this medium were selected[21]. The bacterial isolates were inoculated in the basal medium enriched with chicken feather waste. Native chicken feathers were cut with scissor to small pieces of 1-3 cm long, washed and defatted with chloroform: methanol (1:1 v/v) for 2 days followed by chloroform: acetone: methanol (4:1:3 v/v/v) for 2 days and finally grinded for using in basal medium. The pH was adjusted to 8.0. The medium was incubated in a rotary shaker at a speed of 150 rpm for 37°C for 24 hours. After incubation, the cells were removed by centrifugation at 10,000 rpm for 10 minutes and the supernatant was collected and examined for enzymatic activity.

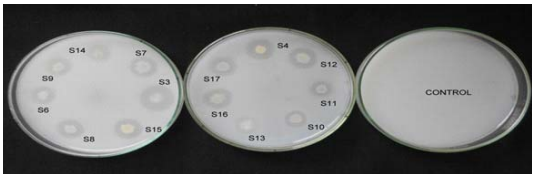

Fig. 1. Keratinolytic activity of the isolates on Skimmed milk agar

Keratinase assay

Keratinase activity was determined spectrophotometrically. The reaction mixture contained 0.1% keratin in 20 ml of 0.1 mol-1 Tris buffer (pH 8) and 40µl of enzyme solution was taken and was incubated for 30 minutes at 55ºC. The reaction was stopped with 500µl 0.1 mol-1 trichloroacetic acid (TCA) in 0.1 mol-1 Tris buffer, pH 8. The amino acid liberated was measured as the absorbance at 540 nm against a reagent blank and the quantity was determined from a standard tyrosine solution[1].

Preparation of Inoculum

A 100 ml nutrient broth solution was prepared and sterilized at 121°C for 20 min. The medium was inoculated under aseptic conditions with bacteria. The broth culture was incubated for 14 hrs on a rotary shaker (150 rpm) at 37°C and was used for inoculating the degradation medium.

Table 1. Degradation percentage and enzyme activity of the isolates

S.No.	Isolates	Initial Weight [Feathers]	Final Weight [After 4 Days]	Degradation (%)	Enzyme Activity (U/ml)
1.	S3	1g	0.20±0.014^{g}	80 ±1.41^{a,b}	11.67±0.057^{a}
2.	S4	1g	0.26 ±0.035^{f,g}	75 ±3.53^{a,b}	9.77±0.029^{b}
3.	S6	1g	0.54 ±0.028^{c,d}	46 ±2.82^{d,e}	4.55±0.084^{g}
4.	S7	1g	0.45 ±0.021^{d,e}	58 ±2.12^{c,d}	5.64±0.127^{f}
5.	S8	1g	0.32 ±0.049$^{e, f, g}$	69 ±4.94^{a,b,c}	8.53±0.056^{d}
6.	S9	1g	0.66 ±0.007^{b,c}	35 ±0.70^{e,f}	3.31±0.035^{h}
7.	S10	1g	0.80±0.028^{a}	20±2.82^{g}	1.83±0.042^{k}
8.	S11	1g	0.80±0.042^{a}	20±4.24^{g}	1.63±0.113^{k}
9.	S12	1g	0.35 ±0.028^{e,f}	65 ±2.82^{b,c}	7.05±0.07^{e}
10.	S13	1g	0.35 ±0.042^{e,f}	65 ±4.24^{b,c}	7.14±0.077^{e}
11.	S14	1g	0.66 ±0.021^{b,c}	35 ±2.12^{e,f}	3.09 ±0.071^{h,i}
12.	S15	1g	0.30 ±0.042^{f,g}	70±4.24^{a}	8.89±0.042^{c}
13.	S16	1g	0.70 ±0.014^{a,b}	30 ±1.41^{f,g}	2.57±±0.028^{j}
14.	S17	1g	0.65 ±0.028^{b,c}	35 ±2.82^{e,f}	2.92±0.056^{i}

Data are means±SD,
Different letters in each column denote significant differences ($p<0.05$, n = 2) according to a Tukey's HSD test.

Bioconversion of poultry feather waste by the isolated strains

For studying the biodegradation of poultry feather waste, the keratinous wastes (chicken feather) was fragmented into pieces with about 1 cm long and added (1% w/v) to the fermentation media as a sole source of carbon and nitrogen. The percent of keratinous waste degradation was determined. The residual feather was washed, dried and scaled to calculate degree of degradation (DD) by using following equation[8]

DD (%) = (TF-RF) ×100/TF

Where, TF is the total feather and RF is the residual feather

RESULTS AND DISCUSSION

In the current study fourteen bacteria were isolated from the soil samples were collected from three different sites i.e. feather waste dump, barber shop and agriculture field from Allahabad, India, the isolates were named S3, S4, S6, S7, S8, S9, S10, S11, S12, S13, S14, S15, S16 and S17. All the isolates (S3 to S17) were screened for keratinolytic activity on the Skimmed milk agar plates (Fig. 1). The organisms producing zone of hydrolysis on Skimmed milk agar plates were considered as keratinolytic organisms. The keratinolytic activity of the isolates (S3 to S17) is mentioned in (Table 1). According to the results, isolate S3 from feather waste dump soil was showing the largest zone of inhibition and maximum enzyme activity was recorded (11.67 U/ml) and was selected as best isolate among all the isolates. Further it was identified as *B.licheniformis* with the help of various biochemical tests mentioned in (Table 2).

Table 2. Identification of the isolate (S5)

Characteristics		Results
Colony Charecteristics	Color	White
	Margins	Undulate
	Opacity	Opaque
Morphological Characteristics	Shape	Rods
	Endospore	+ve
	Gram stain	Gram +ve
	Motility	+ve
Biochemical Characteristics	Indole production	-ve
	Methyle red test	+ve
	Voges-Proskauer test	+ve
	Citrate utilization test	+ve
	Catalase activity	+ve
	Starch hydrolysis	+ve
	Nitrate reduction test	+ve
	Oxidase test	+ve
	Gelatin hydrolysis	+ve
	Caesinase	+ve
	H_2S production	-ve
	Esculin hydrolysis	+ve
	Urease	-ve

Previous studies conducted for the isolation of keratinolytic organism from soil and other natural sources, reported the isolation of keratinase producing strains from *B. subtilis*, *B. licheniformis*, *B. pumilis*, *B. cereus*, *B. halodurans*, *Bacillus weihenstephanensis* and *B. pseudofirmus*[4,11,18,8,17, 19,6] as a potential keratinolytic organism and there possible use in field studies for biodegradation of feather in feather processing units.

Biodegradation of poultry feather waste by the isolated strains was also determined by shake flask method (Table. 1) and according to the results it was found that isolate S3 was showing the highest degradation percentage (80%). Degradation percentage in the present study was found very effective as compared to some of the results reported in previous studies for instance, *Bacillus cereus, B. lichenimormis* and *B. subtilis* caused 78.16%, 74.39% and 73.41% feather degradation respectively[13]. On the other hand some of the reports stated greater degradation of feather as compared to present study for example, *B. pumilus* FH9 was able to degrade 96% and *B. lichenimormis* SA1 hydrolyzed 87.2%[6]. Some isolates have been described completely degrading feathers in culture medium such as *B. megaterium* F7-1[16], *Bacillus pseudofirmus* FA30-01[9].

CONCLUSION

The bacterial isolate *Bacillus licheniformis* showed up to 80% degradation of chicken feather waste. Hence, it could be a potential microbe for commercial application in feather degradation and it can also contribute to efficient solid-waste management, where continuous accumulation of feather wastes poses serious environmental problems.

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