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Bioconversion of Chicken Feather Waste to Eco-friendly Bio-fertilizer: An Agricultural Application

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A B S T R A C T

Indiscriminate use of synthetic fertilizers causes the contamination of the soil, water, and reduced soil fertility. Poultry farming carries a scope for rapid and large profit and become a remunerative business lead to continuously discharge an enlarged quantity of waste such as dead on arrival, feathers, and bones. Feathers are the rich source of keratin proteins and amino acids this factor makes feather applicable to produce of fertilizers and feather meal. The present study aim was to degradation of poultry chicken feathers and its conversion to Bio-fertilizer. Bacteria P3A and BS3B were isolated from feathers waste dumping area and identified as *Staphylococcus epidermidis* and *Bacillus cereus* respectively by the 16S rRNA analysis. It was found that crude enzyme had potential to breakdown the feathers. Bio-fertilizer produced from isolates P3A and BS3B shows 20 cm and 25 cm shoot length and 10 cm and 12.5 cm root length of wheat crop and 16 cm and 18 cm shoot length and 4 cm and 4 cm root length of chick pea crop respectively. Feather bio-fertilizer treated crops showed vigorous growth than the control crops. Keratinase enzyme was useful to convert poultry chicken feather waste into bio-fertilizer.

Key words: Keratinous waste, Keratinolytic bacteria, Keratinase, Bio-fertilizers, Seed germination

In India agriculture field is the fundamental profitable additional factor which supports the financial development of a nation. To maintain growing demand of farming products, synthetic fertilizers and pesticides are adequately used, which affects the human fitness. Tremendous apply of fertilizers may contaminate the soil and underground water with nitrate. Consumption of such a nitrate contains water can be causes the immobility some of hemoglobin in blood (Sharma and Singhvi 2017). Increases in the poultry industry also increases the waste by products such as feathers, soft meal, viscera, bones, dead arrivals need to be manage proper disposable ways (Brandelli *et al.* 2015). The poultry industry used either chemical treatment or steam pressure to manage feather waste and produce feather meal but those methods also liable for damage of vital proteins, amino acids of feathers (Lasekan *et al.* 2013). Feathers are consists of an essential proteins, amino acids and minerals like phosphorus, calcium, nitrogen, potassium, magnesium, copper, manganese, iron, and zinc (Gurav and Jadhav 2013, Kshetri *et al*. 2013). 90% parts of feathers consist of keratin. Keratin is the insoluble recalcitrant in nature and hard to degradation because of the protein molecules are firmly packed and strongly stabilized by several hydrogen bonds and hydrophobic interactions (Brandelli 2008, Kornillowicz-Kowalska and Bohacz 2011).

Some microbial enzymes have ability to degrade insoluble feather keratins, allowing their conversion into feedstuffs, fertilizers, and films (Gupta and Ramnani 2006, Onifade *et al.* 1998). Keratinases are unique proteolytic enzymes that can breakdown insoluble hard keratinous material such as nail, hair, feather and wool. Keratinase enzymes are known to produce by diversity of bacteria, actinomycetes and fungi (Jeong *et al*. 2010, Kornillowicz-Kowalska and Bohacz 2011). The enzymatic breakdown of feather is more useful for preparation of nitrogen fertilizers

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or soil amendments (Kornillowicz-Kowalska and Bohacz 2011). The benefit of using enzyme instead of microorganisms for keratin hydrolysis as it elevated the quantity of amino acids of feather hydrolysate as compared to that obtained by microbial degradation because microbial cells consumed part of the soluble products for their growth (Vesela and Friedrich 2009). Feather hydrolyzate enhances seed germination and growth of plant (Tamreihao *et al.* 2018). This is one of the more economical and environmentally safe methods of recycling the feather. Present study aimed to solve the problem of feather keratin waste of poultry industry. An answer to this havoc is feather degrading bacteria which convert keratin waste to nutritionally valuable feather bio-fertilizer as eco-friendly natural agricultural approach which eventually leads to appropriate human health.

MATERIALS AND METHODS

Isolation of feather degrading bacteria

The soil samples for the isolation of feather degrading bacteria were collected from different locations around Barshi region of Maharashtra from regular poultry feather waste dumping site**.** Soil sample was serially diluted and 1ml sample from the 10^{-5} dilution was enriched in whole feather broth medium for 7 days at 120 rpm. Primary screened on skim milk agar for proteolytic bacteria. Clear zone forming colonies were selected and subsequently inoculated in whole feather broth medium (feather, 5; K_2HPO_4, 0.3; KH_2PO_4 0.4; NaCl, 0.5; pH 7.5 (g/l) aseptically at 120 rpm at 37°C incubated for 7 days.

Identification of feather degrading bacteria

The feather degrading bacteria were identified based on morphological, biochemical characteristics and16S rRNA gene sequencing analysis.

Feather degradation by crude enzyme

Keratinase production was carried out in optimized whole feather broth medium individually and crude enzyme was extracted. Degradation of waste poultry feather by crude enzyme preparation was performed by method described by Abdel-Fattah *et al.* (2018) with some modifications. In brief, the mixture composed of 15 ml of 0.1 Molar potassium phosphate buffer of pH 8 with 0.062 g feather was sterilized and aseptically added the 5 ml crude enzyme and incubated at 37°C at 120 rpm for 96 hrs.

Poultry chicken feather conversion to bio-fertilizers

The feather hydrolysate obtained from breakdown of poultry feather through the action of crude enzyme was used for the preparation of bio-fertilizer. The Carrier material for bio-fertilizer was prepared by applying the methods of Paul *et al.* (2013 and 2018) with some modifications in brief 50 ml feather hydrolysate obtained from action of enzymatic breakdown of chicken feather added to 100 g sterilized soil then dried at 50°C in hot air oven for overnight.

Effect of bio-fertilizer on crop growth

The 100g bio-fertilizer bearing soil was mixed with 200 g sterilized soil and kept undisturbed for 1 day. healthy and approximately equal size seeds of chickpea variety-Vijay (*Cicer arietinum*) and wheat variety Raj-4083 were selected for study. 5 seeds per pot were sown and 21 days pot experiments were carried out in winter season. For Control pot, seeds were sown in sterilized soil without feather bio-fertilizer treatment**.** The physical characters of crops were observed such as seed germination, root length, shoots length. The experiment were conducted in triplicate. The germination was calculated on 7th day based on the common seedling evaluated and it was expressed in percentage using the following formula:

$$\text{Seed germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{Total No. of seeds sown}} \times 100$$

RESULTS AND DISCUSSION

Isolation and identification of feather degrading bacteria

During the preparation of bio-fertilizer from the feather waste isolate P3A was selected from feather dumping site near to Parande road and BS3B from the Naushad poultry farm from Barshi town. Morphological, biochemical and 16SrRNA analysis identification study reveals that isolate P3A was gram positive motile highly similar with *Staphylococcus epidermidis* With the Accession number LC530604 and bacterial isolate BS3B was highly similar with *Bacillus cereus* with Accession number LC530607. Isolate P3A showed 91.1% feather degradation after 72 hrs and BS3B showed 78.5% feather degradation after 96 hrs. It indicates that the feather waste dumping sites are the rich source for the isolation of feather degrading bacteria. Venkata and Divakar (2013) isolated the *Bacillus cerus, Bacillus licheniformis* and *Staphylococcus aureus* from the poultry waste. Arun Kumar *et al.* (2012) in his study recorded the 85% feather degradation in seven days.

Feather degradation by crude enzyme

Visual observation shows that crude enzyme extracted from P3A and BS3B isolate shows maximum breakdown of feather after 72 hrs and 96 hrs in a 0.1M Potassium Phosphate buffer of pH 8 solution respectively (Fig 1). Korkmaz *et al.* (2004) used crude enzyme for feather degradation they found feather barbules were degraded within 3 days at 60°C.

Effect of bio-fertilizer on crop growth

The fertilizer applied pots showed 100% germination after 3 days of sowing. While control pot showed 60% germination.

Table 1 Effect of Feather Bio-fertilizer on crop growth

Agronomics attributes	Agronomics attributes			Chick-pea (gram) crop growth		
	Control	P3A	BS3B	Control	P3A	BS3B
Mean shoot length (cm)	16.5	20	25	12	16	18
Mean root length (cm)	9	10	12.5	4	4	4

Test Control

Fig 1 Enzymatic degradation of feather

Bio-fertilizer

Fig 2 Feather Bio-fertilizer

Control P3A

Fig 3 Effect of P3A Bio-fertilizer on wheat crop

Control BS3B

Fig 4 Effect of BS3B Bio-fertilizer on wheat crop

Fig 5 Effect of P3A bio-fertilizer on chick-pea crop

Fig 6 Effect of P3A bio-fertilizer on chick-pea crop

Pots applied with P3A bio-fertilizer recorded as 20 cm and 16 cm shoot length and 10 cm and 4 cm root length for the wheat crop and chick pea crop respectively (Fig 3&5). While the application of BS3B bio-fertilizer shows 25 cm and 18 cm shoot length and 12.5 cm and 4 cm root length for the wheat crop and chick pea crop respectively (Fig 4&6). Use of feather bio-fertilizer shows more hairy roots in both the crops and more branches and daughter branches observed in chick-pea crops. Similar pot experiment done by Rai *et al*. (2015) and they found enhanced seed germination and growth of Bengal gram in soil treated with feather hydrolyasate. Paul *et al*. (2013) reported that the use of feather hydrolysate improved weight of shoot, number of root hairs, and support the growth of plant. Hadas and Kautsky (1994) reported that feathers shatter gradually and thus act as good quality sources of slow-release fertilizers.

India is agricultural-based nation due to rising population and limited land resource, there is an essential demand for increasing the productivity of an agricultural field (Choi and Nelson 1996, Kim *et al.* 2001) to complete this requirement chemical fertilizer and pesticides are commonly used. An additional food sector poultry industry generates enormous amount of unsafe organic solid waste products including chicken feathers. Even though high nitrogen content chicken feathers cannot be directly applied as organic fertilizer because of the insoluble form of nitrogen (Gorecki *et al.* 2006). Keratinolytic bacterial strain *staphylococcus epidermidis P3A* and *Bacillus cereus* BS3B was isolated from a feather waste dumping location and identified by of 16S rRNA analysis. Studies have revealed that promising isolates produce maximum amounts of keratinase enzyme. Application of feather bio-fertilizers shows vigorous crop growth.

A significant study has been committed to keratinase enzyme, which can breakdown recalcitrant keratins and assign the bioconversion of keratinous material to bio-fertilizers with elevated nutritional values and without any damaging amino acids which boost their biotechnological and industrial applications. Feather degradation property of *staphylococcus epidermidis P3A and Bacillus cereus BS3B* could be competently utilized in chicken feather waste management. The use of feather bio-fertilizer is economic eco-friendly method reduces environmental pollution improve soil texture, agro ecosystem and plant growth.

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