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Enhancement of Soil Nutrition using Fermented Feather and their Efficacy on Seed Germination

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ABSTRACT

Present study includes the biodegradation of feathers into nitrogen rich organic manure by Chrysosporium tropicum and Malbranchea sp. isolated from soil. Protein and amino acids were released due to feather degradation in submerged state fermentation. These degraded feathers used to produce low-cost organic manure for restoration of soil nutrition. Feather composts support better growth for pea and rice seed germination. Bioconversion of feather wastes could be a safe method of recycling by this method. This microbial process, not only solves economic and environmental problems, but at the same time generating value added bio-products with potential industrial and organic farming application.

Key word: *Keratinolytic fungi, Bio fertilizer, Feather Compost*

INTRODUCTION

Typically, each bird has up to 125 gm of feather and with more than 400 million chickens being processed every week worldwide, the daily accumulation of feather waste reaches five million tons[1]. Piling up of this waste material result in accumulation of dumps, causing a global environmental problems for land and underground water resources. Several workers have been reported keratin degrading activity of various organisms' actinomycetes[2] and fungi[3]. The limitations of conventional methods for producing readily digestible feather meal impel the use of microbial treatment of recalcitrant feather wastes as ecologically safe, low-cost method that offers mild reaction conditions[4]. Bioconversion of keratin residues is attracting increasing biotechnological interest since it might represent an alternative way of waste management that could be coupled with the production of valuable products[5,6].

The protein rich concentrates feather meal generated for poultry feed can also be applied to organic farming as a semi slow release nitrogen fertilizer[7,8]. Feather meal being nitrogen rich (15% N), inexpensive and readily available sources serves as a potential substitute.

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Most appropriate application of recycled keratin wastes and other organic wastes is as cheap soil amendments and fertilizers, providing organic matter, an important constituent of biologically active and productive soils[9]. Moreover, using organic amendments may result in a soil with greater resistance against plant[10] pathogenic organisms and reduced use of fungicides. Biofertilizers are the formulation of living microorganisms, which are able to fix atmospheric nitrogen in the available form for plants either by living freely in the soil or being associated symbolically with plants[11] and keep the soil environment rich in all kind of micro and macro nutrients via nitrogen fixation, phosphate and potassium solubilisation or mineralization[12].

India, second largest populous country, mostly depends on agriculture for a livelihood. Nitrogen is required in large quantities for plants to grow, is provided in the form of synthetic chemical fertilizer as urea. After water, nitrogen is the second limiting factor for plant growth in many fields and deficiency of this element is met by fertilizers[13]. Such chemical fertilizers pose a health hazard and a microbial population problem in soil besides beings quite expensive and making the cost of producing high. Excessive use of chemical fertilizer not only cost intensive but also creates environmental problem. In such a situation the biofertilizers play a major role[14]. Agricultural importance of biofertilizer in rice and pea seed is directly related to their ability to fix nitrogen and other positive effects of plants and soil. Therefore to develop ecologically friendly methods for more effective utilization of keratin wastes to obtain new organic amendments and fertilizers for improving quality of agricultural soils.

MATERIALS AND METHODS

Isolation and screening of keratinolytic fungi

Fungi were isolated by hair baiting method and identified by morphological examination. Fungi were screened by method of Chao[15] on skimmed milk agar and keratin powder agar plates for solid screening and incubated for 3, 6, 9 and 12 days for 28˚C. The diameter of the clearing zone was measured to quantify activity.

Degradation of feather in submerged state formation

The media was prepared by method of Kumar[16]. The flasks containing media were inoculated with a disc (6 mm diameter) of culture. The three control flasks were run as keratin controls to which were added 50 ml of basal medium and 0.2 g of feathers, fungus controls to which were added 50 ml of basal medium and fungal inoculum and test sample to which were added 50 ml of basal medium 0.2 g feathers and fungal inoculum. Each flask was incubated at 28 $\pm 2^0$C for 4, 8, 12, 16, 20 days.

Estimation of protein release

The filtrate was collected and centrifuged at 5000 rpm for 5 min. the supernatant was collected and used for estimation of protein release by the method of Lowry[17].

Estimation of amino acids

Release of amino acids was monitored following the method McGrath[18].

Preparation of feather compost and feather meal

Feather meal and compost was developed by modified method of Choi[8]. Feathers were washed with tap water and detergent, air dried and autoclaved 200g of soil in a plastic box. Moist the soil with autoclaved distilled water. 5g autoclaved feather was mixed with soil and the preparation was inoculated with 20ml of aqueous spore suspension of culture and incubated for 15 -30 days.

Estimation of nitrogen content

The nitrogen content of compost was measured at 0, 15 and 30 day of interval with Nice soil testing kit. Three samples of feather compost were taken from 0 day interval (control), 15 day interval, and 30 day. The developed colour is compared with Nitrogen Colour chart.

Effect of feather compost and feather meal on seed germination

Six pots were taken and filled with soil. The pots were marked as A, B, C, D, E. The seedling of rice was transferred to pots. In pots A feather meal was added, B feather compost, C *Chrysosporium tropicum*, D *Malbranchea* sp., E urea and F no supplements were added respectevely. The pots were watered every day and analysis of plants was done on regular interval of day.

RESULTS AND DISCUSSION

Isolation and screening of keratinophilic fungi

Hair baiting method was used for the isolation of keratinophilic fungi. Five isolates were obtained from six soil samples. Isolated keratinophilic fungi were tested on skimmed milk agar plates and keratin powder agar plates to test their keratinolytic activity. Maximum clearing zone was made by fungus *Chrysosporium tropicum* (7mm), *Malbranchea* sp. (2mm), while *Aphanoascus fulvescens* (1mm), *Chysosporium keratinophillum* (1mm) was found moderate and fungus *Fusarium oxyspoum* show no zone. Similar clearing zone were observed by Chao[15] and Kumar[19].

Degradation of feather in submerged state fermentation

Feathers were partially degraded due to fungal action. Protein and amino acids are released in medium.

Estimation of protein release

The *Chrysosporium tropicum* and *Malbranchea* sp. produced inducible keratinase by feather degradation and degrade feather incompletely. The protein content released in supernatant due degradation of feather is determined (Table 1).

Estimation of amino acids content

The release of different amino acids by *Chrysosporium tropicum* and *Malbranchea* sp. (Table 2). Maximum amount of cysteine, lysine, methionine and valine were released by *Chrysosporium tropicum and Malbranchea* sp at 20^{th}, 4^{th}, 4^{th}, 4^{th} and 20^{th}, 12^{th}, 8^{th}, 20^{th} day of incubation period, respectively (Table 3). The minimum amount of cysteine, lysine, methionine and valine were released by *Malbranchea* sp at 8^{th}, 8^{th}, 20^{th}, 8^{th}day of incubation. Kumar[20] obtained maximum amount of lysine and cysteine was released by *Acremonium strictum* 18.00 µg/ml and 32.00 µg/ml.

Development of feather compost and feather meal

Biodegradation of feathers were observed in 30 days. During composting process the keratinous material were observed for morphological changes occurred due to the colonization of fungus Fig. 1 (a & b). The isolate intensely degraded feathers under exposed, submerged and in soil conditions and emerged as a potential keratinolytic organism suitable for industrial applications as well as for feather composting purpose[21]. Nayaka[22] used feather meal as a source of nitrogen to plants, feather meal is used in formulated animal feed and in organic fertilizer. Although total nitrogen levels are fairly high (up to 12%), it is a source of slow–release organic, high nitrogen fertilizer. It can be used to increase green leaf growth, activate compost decomposition, and improve soil structure.

Estimation of nitrogen content

The nitrogen content of compost is estimated by Nice Soil Testing kit at 0 (control), 15 & 30 days interval. The colour was developed (Fig. 2b) is compared with Nitrogen colour chart (Fig. 2a) and recorded the result. At 30^{th} day the feather compost contain high nitrogen content as compared to 15^{th} day and 0 day. Nitrogen is one of the important primary nutrients, serves as the essential component of amino acids, the basic structural units of protein[22,23].

Effect of feather meal and feather compost on seed germination

The effect of feather compost, feather meal and keratinophilic fungus on the growth of paddy was established on the basis of growth of control and treated plants as shown in Fig. 3(a) and (b). The effect of feather compost, a feather meal and keratinophilic fungus was observed in significantly different. The growth of pea seeds was E> C> A> B> D. Addition of feather lysate to soil has been shown to

increase urease and protease enzyme activity. Bomke[24] observed that poultry manure had highest effect of nitrogen on the growth of plant and similar result reported on the growth of *C. roseus*. Proteases and ureases in soil play a significant role in nitrogen mineralization, an important process in regulating the amount of plant available nitrogen for plant growth[25]. Feathers being rich in protein their microbial degradation in soil would release peptides and amino acids along with other products which may stimulate soil microbial activity there by facilitating the assimilation of nutrients by plants[26] and better growth.

Table 1: Determination of Total protein released (µg/ml) in supernatant fluid

	INCUBATION PERIOD (DAYS)				
	4 DAY	**8 DAY**	**12 DAY**	**16 DAY**	**20 DAY**
			Chrysosporium tropicum		
CONTROL	003.20	000.00	000.40	000.20	001.30
KERATIN CONTROL	168.30	199.60	199.40	192.20	188.00
TEST SAMPLE*	256.40	209.40	210.20	211.50	212.20
TOTAL PROTEIN**	424.70	409.00	409.60	403.70	400.20
			***Malbranchea* sp.**		
CONTROL	008.70	001.90	005.00	000.00	002.00
KERATIN CONTROL	139.19	121.30	118.13	116.71	112.50
TEST SAMPLE*	159.02	161.32	158.12	148.70	148.50
TOTAL PROTEIN**	298.21	282.62	276.25	265.41	261.00

Abbreviations: - * = mean of 3 readings, ** = total protein content (keratin control + mean of 3 readings).

Table 2: Determination of amino acids (µg/ml) in supernatant fluid

Amino Acid	**Fungi**	**Release of Amino Acids (Incubation period in days)**				
		4	**8**	**12**	**16**	**20**
Cysteine	*Chrysosporium tropicum*	20.00	21.20	20.53	19.00	23.24
	Malbranchea sp.	16.00	15.92	16.32	17.25	17.98
Lysine	*Chrysosporium tropicum*	14.00	13.09	13.02	13.06	12.98
	Malbranchea sp.	7.05	6.98	7.98	7.02	7.25
Methionine	*Chrysosporium tropicum*	9.00	8.78	7.06	8.00	8.56
	Malbranchea sp.	6.95	7.00	6.23	6.72	6.21
Valine	*Chrysosporium tropicum*	8.02	7.98	7.55	7.92	7.32
	Malbranchea sp.	5.28	4.98	5.75	5.20	5.13

Absorbance was read at 365nm, 600nm, 480 nm and 460 nm. ****=** mean of 3 samples + keratin control

Fig. 1: Development of (a) feather compost (b) feather meal

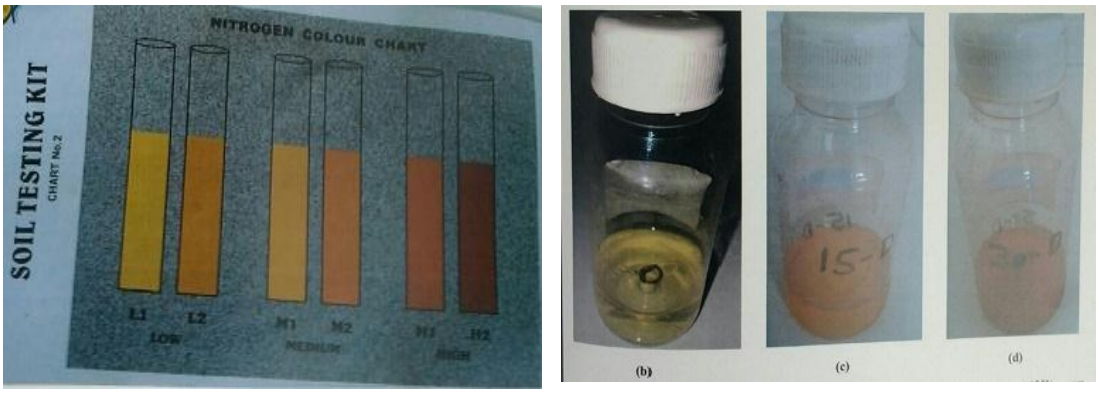

Fig. 2: Reference color chart for N_2 Content (a)0 d; (b) 15 d; (c) 30d

Fig. 3: (a & b) *in vitro* expermints on paddy and pea seed germination

CONCLUSION

Five isolates were obtained from poultry farms and were screened for keratin degradation. *Chrysosporium tropicum* and *Malbranchea* sp. were selected for maximum keratinase production. Supernatant were used to study release of protein and amino acids. Enzymatic degradation of feather by *Chrysosporium tropicum* and *Malbranchea* sp. revealed that *Chrysosporium tropicum* and *Malbranchea* sp released maximum amount of protein 424.70(µg/ml), 298.21 (µg/ml) respectively. In crude enzyme moderate amount of amino acids were also estimated maximum amount of amino acids were produced by *Chrysosporium tropicum* as compared to *Malbranchea* sp. Feather composts support better growth for pea and rice seed germination. Bioconversion of feather wastes could be a safe method of recycling by this method. This microbial process, not only solves economic and environmental problems, but at the same time generating value added bio-products with potential industrial and organic farming application.

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