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Determination of feather degradation in soil by non dermatophytic fungi using singly and in combination

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ABSTRACT

Keratin azure and feather degradation in soil determines the amount of keratin degradation during the activity of different fungi in different combinations in soil. The aim of this study is to evaluate degree of feather degradation in soil by non dermatophytic fungi acting singly and in combination. Fungi frequently occurring in soil were selected for hen feather and keratin azure degradation *in vitro* individually and in combination in different sets in soil. It is concluded that 20 fungi tested showed feather and keratin azure degradation in culture. Five selected fungi *Acremonium implicatum, Chrysosporium queenslandicum, Chrysosporium pannicola, Malbranchea pulchella* and *Verticillium lecanii,* when used in combination with *Chrysosporium keratinophilum* showed feather and keratin azure degradation. Further feather and keratin azure degradation was monitored in soil when these were used individually and in combination with *C. keratinophilum* and almost above 90% degradation was recorded. The determination of amount of keratin degradation in soil by using individual fungus and fungi in combination is of immense importance in order to find out how much keratin is degraded in soil. The measurement of feather and keratin azure degradation in soil by using five fungi singly and in combination with *C. keratinophilum* presented here for the first time.

INTRODUCTION

Keratin rich wastes in the form of feathers are highly available as by products of agro industrial processing. Increased needs for energy conserving and recycling have stimulated the researches for alterations for the management of recalcitrant keratinous wastes. Keratinases of soil fungi shows potential use in biotechnological processes involving keratin hydrolysis. These enzymes are useful in processes related with bioconversion of keratin waste in to feed and fertilizers. Biodegradation of intensively produced animal waste is now viewed as an alternative avenue for creating a viable end product. The composting process comprises multiple biochemical transformations in which microorganisms grow in compost material. It is also closely connected with the formation of bio films in which degradation is more efficient.

The large amount of feathers produced and their localized accumulation create a serious disposal problem leading to environmental pollution. The feather occumulation in natural settings with large bird population suggests the existence of great number of keratinolytic microorganisms. Chicken feather waste

CFW is mainly keratin which is not normally degraded by common proteolytic enzymes. The keratin degradation is accomplished by the production of keratinase. The keratin degradation in submerged cultures is now studied by several workers using different fungi and different types of keratinous substrates. There appears very few determinations which shows keratin degradation in soil and also methods of keratin degradation in soil are not available except that of Wainwright[6]. In natural soil where keratin degradation occur microorganism do not act singly. A combination of different microorganism play deciding role in complete degradation of keratin making the soil enriched with their end products.

With this in view fungi frequently occurring in soil were selected for hen feather and keratin azure degradation *in vitro* individually and in combination in different sets in soil. Keratin azure degradation and modified method using feather degradation in soil determines the amount of keratin during the activity of different fungi in different combinations in soil. The aim of this study is to evaluate degree of feather degradation in soil and in submereged cultures of non dermatophytic fungi acting singly and in combination.

EXPERIMENTAL

Material

The keratinophilic fungi were isolated from soil by baiting method using feathers as bait. These were grown and cultured on potato dextrose agar. Feather degradation in submerged cultures was determined by monitoring feather and keratin azure loss and keratinase production by using 20 fungi (TABLE 1). *Acremonium implicatum, Chrysosporium queenslandicum, C. keratinophilum, C. pannicola, Malbranchea pulchella* and *Verticillium lecanii* were used individually and in different set with combination of *C. keratinophilum* (TABLE 2 & 3).

Keratinase determination

Fungal strains were tested in submerged culture. The feathers were cut in to 1-2 cms pieces, washed and dried. The fermentation medium was prepared according to Friedrich et al.[2] with some modifications. It contained hen feathers 20g, $MgSO_4.7H_2O$ 0.5g, $FeSO_4.7H_2O$ 0.01g and $ZnSO_4.7H_2O$ 0.005g in phosphate buffer (0.028 mol, pH 7.8) per liter of medium. An aliquate of 50 ml of the solution of mineral salts in the buffer were poured in to 250 ml Erlenmeyer flasks. Fungal inoculum was prepared by suspending spores from the tube of above mineral agar slant culture in 15 ml sterile water. Five ml of this suspension was taken to inoculate one flask. The flasks in triplicate were inocubated at 28±2°C for 14 days. Prior to analysis, the fermentation broth was filtered. Keratinase production was measured using the method of Takiuchi et al.[5]. Keratin powder was incubated with 3.0ml phosphate buffer (0.028 mol^{-1}, pH 7.8) and 2.0 ml culture filtrates for 1 h at 30°C in water bath. The enzyme reaction was stopped by adding 2.0ml of 10% trichloracetic acid and samples were cooled at 4°C for 30 minutes. They were then centrifuged for 15 minutes at 10,000g in a refrigerated centrifuge. The absorption of the supernatant fluied at 280nm was measured spectrophotometrically. The blank was treated in the same way except for the addition of TCA which was accomplished before the enzyme reaction. An increase of 0.100 absorbance was taken to indicate one unit of enzymatic activity.

Keratin azure degradation

Microbial degradation of keratin azure *in vitro* leads to the release of blue color into the medium which can be easily measured spectrophotometrically. The amount of dye lost from a known weight of keratin azure following incubation in soil is determined. Keratin azure (0.01g) was placed between 2 squares of polyster fabric (4×4cms) of mesh size 4 μm, which exclude soil animals but not microorganisms. The edges of the material heat sealed. The mesh bag was placed inside a plastic photographic slide frame (55cms). The two halves of the slide frame were then pressed together in order to seal them. Slides with mesh bags were placed in 200g of soil in triplicate and incubated in sterile polythene bags at 20% v/w water content at 28±2°C. The polythene bags were closed with elastic so as to leave small hole to allow gaseous exchange. These were also placed in soil which has been autoclaved on 3 successive days at 120 lbs for 30 minutes to act as controls.

After incubation in soil the slide frames were removed and opened and any undegraded, residual kera-

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TABLE1 : Feather and keratin azure degradation and keratinase production by non dermatophytic fungi

	Ku/ml	FL%	AL%
Acremonium implicatum	190.2	17.6	20.5
Aspergillus quercinus	60.5	14.3	6.4
Aspergillus ustus	60.5	14.2	6.2
Botryotrichum keratinophilum	110.4	20	12.5
Chaetomium globosum	30.4	15.2	7.5
Chrysosporium queenslandicum	160.5	30.5	15.2
Chrysosporium tropicum	92.4	18.5	9.5
Chrysosporium indicum	85.9	18.5	7.8
Curvularia indica	140.4	20.5	12.5
Keratinophyton terreum	118.7	36.7	20
Malbranchea pulchella	170.2	37.2	16.5
Penicillium lilacinum	158.4	15.8	8
Trichoderma harzianum	60.3	14.4	7.5
Trichoderma hematum	58.6	13.3	6.2
Verticillium lecanii	195.4	25.2	22.2
Chrysosporium keratinophilum	200	75	25.5
Chrysosporium pannicola	150.5	19.6	22.6
Chrysosporium crassitunicatum	145	19.4	14.2
Chrysosporium sulphureum	80.4	19.9	12.8
Chrysosporium zonatum	65.5	18.5	10.5

Ku/ml=Keratinase, FL%=Feather Loss, AL%= Keratin Azure Loss

tin azure was removed from the inside surface of mesh bags and collected. The residual substrate was repeatedly washed and centrifuged until no further dye released occurred. The water was then removed and 10 ml of NaOH (10%w/v) added. Finally the tube was heated in a boiling water bath until the keratin azure dissolved when the resultant blue color was determined spectrophotometrically at 595 nm, using NaOH as blank. The 0.01g keratin azure was then solubilized in the same way to determine the amount of dye initially present in the substratum, so that the amount of dye lost could be calculated by subtraction.

Feather degradation

In place of keratin azure 200mg of washed and sterilized hen feathers were also placed in between squares of polyster fabric in the similar manner as described earlier for keratin azure and residual undegraded feather was removed and feather degradation was calculated in case of individual fungi and in combination with *C. keratinophilum* in soil.

RESULTS AND DISCUSSION

The results of feather degradation and keratin azure degradation revealed that all the 20 fungi used in this study are highly keratinolytic which is also supported by their keratinase production (TABLE 1-3). *C. keratinophilum* showed maximum keratinase, feather and keratin azure degradation. *A. implicatum* was next to it and showed 190.2 ku/ml keratinase, 17.6% feather degradation and 20.5% keratin azure degradation and eight fungi produced more than 100ku/ml keratinase. The minimum keratinase produced was 58.6ku/ml by *Trichoderma hematum*. The rest of the fungi showed more than 50ku/ml keratinase.

When *A. implicatum, C. queenslandicum, V. lecanii* and *C. pannicola* were grown with *C. keratinophilum* independently the enhanced keratin azure degradation was noted during 2, 3 and 4 weeks duration. Keratin azure degradation was found maximum in *C. keratinophilum + V. lecanii* set showing 75% keratin azure degradation. Next to it were *C. keratinophilum + A. implicatum* showing 75% keratin azure degradation. *C. keratinophilum + C. pannicola* set showed 60% keratin azure degradation. When all these 6 fungi were grown in together the 80% keratin azure degradation was noted.

Feather and keratin azure degradation was monitored by these fungi acting independently up to 4 weeks. Feather degradation in soil was more than 90% after 4 weeks in case of all these fungi and keratin azure degradation was also between 85-100%. When these fungi were used in combination with *C. keratinophilum*, maximum feather degradation, 40.8% was in C. *keratinophilum + A. implicatum* set in 1 week which increased up to 97% at he end of 4 weeks. Similarily keratin azure degradation was 26% at 1 week and 92% at 4 weeks. However, in rest of the cases 100% feather degradation and more than 95% keratin azure degradation was noted. All these six fungi when used in combination exhibited 45.8% feather degradation and 22% keratin azure degradation in 1 week which increased to 100 and 90% respectively in 4 weeks.

The data on keratin measurements of feather degradation in soil are not available however, Mushin and Hadi[4] studied feather degradation by using *A. flavus*,

TABLE 2 : Feather and keratin azuredegradation in soil by non dermatophytic fungi acting singly

	Week	1	2	3	4
Acremonium implicatum	FL	42.9	64.4	88	90.2
	AL	20	40	70	90.2
Chrysosporium queenslandicum	FL	23.75	68.85	87.25	91.4
	AL	15	35	65	85.5
Malbranchea pulchella	FL	44.5	70.2	88.75	93.7
	AL	16	38	70	88.2
Verticillium lecanii	FL	13	58	72.5	92.6
	AL	22	45	74	92.4
Chrysosporium pannicola	FL	37.4	60.5	84.9	91.4
	AL	22	38	68	85
Chrysosporium keratinophilum	FL	21.5	65	82.25	93.7
	AL	25	45	75	100

FL=Feather Loss%, AL=Keratin AzureLoss%

TABLE 3 : Feather and keratin azure degradation in soil by non dermatophytic fungi acting in combination with

	Week	1	2	3	4
Chrysosporium keratinophilum + Acremonium implicatum	FL	40.8	75.4	92	97
	AL	26	45	75	92
Chrysosporium keratinophilum + Chrysosporium queenslandicum	FL	41.2	70	89	100
	AL	16	38	70	90
Chrysosporium keratinophilum + Malbranchea pulchella	FL	30	75.2	93	100
	AL	15	50	72	95
Chrysosporium keratinophilum + Verticillium lecanii	FL	30	75.2	93	100
	AL	15	50	72	95
Chrysosporium keratinophilum + Chrysosporium pannicola	FL	42	80.4	92.8	100
	AL	26	60	80	96
All the fungi	FL	45.8	80.2	96	100
	AL	22	48	76	90

FL=Feather Loss%, AL=Keratin Azure Loss%

C. pannicola, Microsporum gypseum and Trichophyton mentagrophytes and found that *C. pannicola* could be able to cause15, 45 and 64% degradation of feather, wool and hair respectively. The degradation ability of chicken feathers by *Chrysosporium* indicated that these fungi are the most active in degrading feathers. Some non dermatophytic fungi such as *Aspergillus flavus* showed very large amount of keratinase (781 ku/ml) in 10 days[2]. These workers also found *Alternaria radicina, Trichurus spiralis, Stachybotrys atra* to produce 374, 278 and 225 ku/ml keratinase respectively. Marcandes et al.[3] isolated *Aspergillus, Acremonium, Alternaria, Beuvaria, Curvularia, Paecilomyces and Penicillium* which were able to produce keratinase when feather powder was as substrate. Recently non pathogenic *Trichoderma atroviride* found to completely degrade chicken feather[1].

It is concluded that all the 20 fungi tested showed high keratinase activity and feather and keratin azure degradation in culture. Five selected fungi *A. implicatum, C. queenslandicum, M, pulchella, V. lecanii,* and *C. pannicola* when used in combination with *C. keratinophilum* showed increased feather and keratin azure. Further feather and keratin azure degradation was monitored in soil when these were used individually and in combination with *C. keratinophilum* almost above 90% degradation was recorded. The keratin degradation in soil takes place by not a single organism but several others are also involved in the process. Therefore it was thought that if some actively keratin degrading fungi are added in the process these may inhance the activity in culture as well as in soil. The measurement of feather and keratin azure degradation in soil by using five fungi singly and in combination with *C. keratinophilum* presented here for the first time.

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