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Note

A survey of keratinophilic fungi as a tool for hen feather utilization

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One hundred keratinophilic fungi belonging to *Acremonium*, *Aphanoascus*, *Amauroascus*, *Botryotrichum*, *Chrysosporium*, *Ctenomyces*, *Gymnoascus*, *Malbranchea*, *Microsporum*, *Narashimella* and *Verticillium* were tested for their potential to utilize hen feathers as keratinous waste. *Acremonium* sp. 2 and *Chrysosporium europae* released 494.33 and 457.33 μg/ml protein from feathers respectively. *Microsporum gypseum* released 698.66 μg/ml protein from feathers. Some of these keratinophilic strains other than *Microsporum* may prove beneficial as tools for rapid utilization of not only feathers but other hard keratinous substrates and their end product may be of industrial importance.

Key Words——keratinophiles; keratinous waste; utilization.

Keratinophilic fungi and dermatophytes are a highly specialized group of fungi that, through long evolutionary processes, have become adapted to invade, colonize and nourish themselves on the keratinized tissues of men and animals. They are known to thrive and multiply in the keratinized tissues of human and animal. Sufficient data has been accumulated in the past few years on the ecology and distribution of such fungi from India and other countries (Ajello, 1960; Mercantini et al., 1980; Garg et al., 1985; Jain et al., 1985; Dixit and Kushwaha, 1990; Nigam and Kushwaha, 1993).

In general, keratinophilic fungi inhabit a variety of sites frequented by animals and man such as playgrounds, recreation centres, poultry farms, breeding farms, zoological parks and swimming pools. The distribution of pathogenic and non-pathogenic keratinophilic fungi (Ainsworth and Austwick, 1973; Ajello, 1974; Philpot, 1978; Garg et al., 1985) indicates that soil acts as reservoir for primary infection, at least for some pathogenic fungi, and also for those which are potentially pathogenic to animals including men and various pets (Alteras et al.,1984). Besides soil, birds' feathers and nests (Sur and Ghosh, 1980), hairs of wild animals (Salebiau and Carlas,1980), house dust (Nigam and Kushwaha, 1992), water (Simordova and Hejtmanek, 1970), plant debris (Currah, 1985) and dung (Currah, 1985) are other habitats for the survival of potential dermatophytes.

Keratinophilic fungi are able to degrade keratinous substrates completely or partially. Under natural conditions, keratinized tissues are less suitable for microorganisms and only certain highly specialized species of fungi are able to attack keratinous substrates (Nigam and Kushwaha, 1992). When keratinophilic fungi are cultured in media containing keratin, they degrade and may use this compound as a source of carbon and nitrogen. Degradation is accompained by alkalization of the medium and by the high activity of proteolytic exoenzymes. However, most of the fast-growing keratin-decomposing fungi have not been tested for their potential to degrade keratinized waste, particularly hen feathers generated by chicken meat production (Kushwaha, 1998). The fungi tested here belong to the genera *Acremonium* Link, *Aphanoascus* Zukal, *Amauroascus* Schroeter, *Botryotrichum* Saccardo & Marchal, *Chrysosporium* Corda, *Ctenomyces* Ediam, *Gymnoascus* Baranetzky, *Malbranchea* Saccardo, *Microsporum* Gruby, *Narashimella* Thirumalachar & Mathur, and *Verticillium* Nees. They were isolated from potted plant soil by a hair-baiting method, and most of them were deposited in Germplasm Centre for Keratinophilic Fungi (GPCK), Department of Botany, Christ Church College, Kanpur, India; Indian Type Culture Collection (ITCC) New Delhi, India; Microbial Type Culture Collection (MTCC) Chandigarh, India and in the culture collection of Faculty of Medicine (FMR), University of Barcelona, Reus, Spain for reference.

Hen feathers were washed with sterilized distilled water, cut into 2-cm-long pieces, sterilized in an autoclave for 10 min at 15 lb pressure and used as substrate. A mineral medium containing 1.5 g of K_2HPO_4, 0.25 g of $MgSO_4$, 0.005 g of $ZnSO_4$. $7H_2O$, 0.025 g of $CaCl_2$, 0.005 g of $FeSO_4 \cdot 7H_2O$, and 30 g of dextrose per litre of distilled water (pH 6.5) was used in all the experiments. Inoculum was the conidial suspension from the surface of 6 d old colonies on the mineral medium. The conidial suspension was obtained from culture tubes by brushing conidia in 5 ml of sterilized distilled water and 2 ml of conidial suspension was added to each flask. Each

Table 1. Weight loss and protein released from bird feathers during growth of keratinophilic fungi.

S. no.	Fungi tested	Accession no.	Test sample [μg/ml]	Fungus + keratin control[a] [μg/ml]	Total protein[b] [μg/ml]	Weight loss[c]
1.	*Acremonium* sp.1	GPCK 506	135.33± 18.58	231.66±47.35	366.99	29.50
2.	*Acremonium* sp.2	GPCK 537	309.49±185.30	185.33±10.59	494.33	48.00
3.	*Acremonium* sp.3	GPCK 538	192.00± 81.61	249.33±43.87	441.33	32.00
4.	*Acremonium* sp.4	GPCK 539	230.66± 90.52	187.00±27.40	417.66	64.00
5.	*Acremonium* sp.5	GPCK 540	140.00± 22.00	172.33± 5.68	312.33	37.33
6.	*Acremonium* sp.6	GPCK 541	193.00± 6.24	158.00±25.90	351.00	65.00
7.	*Acremonium* sp.7	GPCK 542	126.66± 21.50	184.33±15.80	313.99	24.00
8.	*Acremonium* sp.8	GPCK 543	125.33± 13.42	144.33±21.20	269.66	49.00
9.	*Acremonium* sp.9	GPCK 544	73.33± 1.15	142.00± 1.00	215.33	84.00
10.	*Acremonium* sp.10	GPCK 545	79.00± 10.39	134.66±12.70	213.66	21.00
11.	*Acremonium* sp.11	GPCK 546	83.33± 19.65	113.66± 7.50	196.99	50.00
12.	*Acremonium* sp.12	GPCK 547	62.00± 21.21	127.66± 6.65	189.66	50.00
13.	*Acremonium* sp.13	GPCK 548	14.30± 7.80	72.66± 1.52	87.32	8.99
14.	*Acremonium* sp.14	GPCK 549	61.66± 6.50	86.33± 6.11	147.99	36.00
15.	*Acremonium* sp.15	GPCK 550	31.66± 10.06	76.00± 1.00	107.66	15.00
16.	*Acremonium* sp.16	GPCK 551	17.00± 6.00	89.00± 6.50	106.00	12.00
17.	*Acremonium* sp.17	GPCK 640	54.00± 29.20	104.33± 6.08	158.33	18.00
18.	*Acremonium implicatum*	FMR 6212	50.00± 1.00	137.33± 8.62	187.33	46.00
19.	*Acremonium hennebertii*	FMR 6213	81.33± 40.00	114.00± 4.58	195.33	25.00
20.	*Acremonium hennebertii*	FMR 6214	63.00± 30.51	122.66±23.62	185.66	70.00
21.	*Aphanoascus fulvescens*	GPCK 641	79.66+ 79.75	158.00±13.00	437.66	89.66
22.	*Aphanoascus fulvescens*	GPCK 610	318.00± 91.09	365.66± 7.23	683.66	91.00
23.	*Aphanoascus terreus*	GPCK 642	132.66± 30.74	163.33± 7.50	295.99	48.33
24.	*Amauroascus kuehnii*	FMR 4053	150.33± 29.50	189.33± 9.29	339.66	71.00
25.	*Botryotrichum piluliferum*	GPCK 643	223.66± 64.65	296.33±74.76	519.99	73.00
26.	*Chrysosporium europae*	FMR 300	220.00± 38.21	237.33±28.67	457.33	89.00
27.	*Chrysosporium cuniculi*	GPCK 673	132.66± 21.93	99.00± 6.08	231.66	56.00
28.	*Chrysosporium indicum*	GPCK 502	53.00± 23.57	79.00± 1.00	132.00	11.00
29.	*Chrysosporium indicum*	GPCK 639	34.66± 20.81	76.33± 1.52	110.99	8.00
30.	*Chrysosporium indicum*	GPCK 648	70.00± 14.52	84.33± 3.78	154.33	20.00
31.	*Chrysosporium indicum*	GPCK 649	169.33± 6.35	131.00± 1.00	300.33	18.00
32.	*Chrysosporium indicum*	GPCK 650	86.33± 10.40	109.66±21.73	195.99	12.00
33.	*Chrysosporium indicum*	GPCK 651	129.33± 11.01	99.00± 7.81	228.33	25.66
34.	*Chrysosporium indicum*	GPCK 652	150.66± 3.05	144.33± 6.24	294.99	34.00
35.	*Chrysosporium indicum*	GPCK 653	40.33± 4.04	83.66± 3.21	123.99	19.00
36.	*Chrysosporium indicum*	GPCK 654	79.00± 23.57	77.00± 1.73	156.00	20.00
37.	*Chrysosporium indicum*	GPCK 655	131.66± 6.02	109.66± 1.52	241.32	15.00
38.	*Chrysosporium indicum*	GPCK 656	71.33± 35.11	102.33± 9.71	173.66	40.00
39.	*Chrysosporium indicum*	GPCK 657	32.66± 6.65	74.00± 2.00	106.66	12.00
40.	*Chrysosporium indicum*	ITCC 4730	74.00± 11.53	110.66± 9.45	184.66	21.00
41.	*Chrysosporium keratinophilum*	GPCK 501	96.66± 11.50	89.33± 6.11	185.99	38.66
42.	*Chrysosporium keratinophilum*	GPCK 614	247.33± 46.69	137.00±11.53	384.33	78.32
43.	*Chrysosporium keratinophilum*	GPCK 658	161.33± 1.15	255.66±11.93	416.99	52.33
44.	*Chrysosporium keratinophilum*	GPCK 659	62.33+ 12.50	89.33+ 2.64	151.66	14.32
45.	*Chrysosporium keratinophilum*	GPCK 660	60.66± 28.02	77.00± 2.00	137.66	20.00
46.	*Chrysosporium keratinophilum*	GPCK 661	249.00± 19.05	148.33± 6.65	397.33	81.00
47.	*Chrysosporium keratinophilum*	GPCK 662	202.66± 11.93	143.00± 3.46	345.66	73.00
48.	*Chrysosporium keratinophilum*	GPCK 663	85.33± 12.50	87.66± 1.52	172.99	18.00
49.	*Chrysosporium keratinophilum*	GPCK 773	32.66± 14.22	75.33± 3.51	107.99	21.00
50.	*Chrysosporium keratinophilum*	ITCC 4729	91.66± 12.70	88.00± 2.00	179.33	26.00

51. *Chrysosporium keratinophilum*	P 318	65.00± 23.30	76.33± 1.52	141.33	11.00
52. *Chrysosporium pannicola*	GPCK 612	65.33± 17.24	75.33± 0.57	140.66	23.00
53. *Chrysosporium pannicola*	GPCK 670	150.66± 18.47	203.66±47.18	354.32	11.00
54. *Chrysosporium pannicola*	GPCK 671	41.33± 13.05	73.00± 2.00	114.33	15.00
55. *Chrysosporium pannicola*	GPCK 672	94.00± 00	85.00± 6.00	179.00	31.00
56. *Chrysosporium pseudomerdarium*	GPCK 674	88.33± 36.50	126.66±12.42	214.99	63.00
57. *Chrysosporium queenslandicum*	ITCC 4731	53.66± 17.78	84.00± 3.46	137.66	17.00
58. *Chrysosporium queenslandicum*	GPCK 664	108.00± 8.88	88.66± 5.68	196.66	42.00
59. *Chrysosporium queenslandicum*	GPCK 665	62.66± 11.67	83.33± 3.51	145.99	31.00
60. *Chrysosporium queenslandicum*	GPCK 666	74.00± 2.00	85.00± 2.00	159.00	13.00
61. *Chrysosporium queenslandicum*	GPCK 667	101.66± 12.50	109.00± 3.00	210.66	45.00
62. *Chrysosporium queenslandicum*	GPCK 668	121.00± 1.00	96.00± 3.00	217.00	49.00
63. *Chrysosporium queenslandicum*	GPCK 669	73.33± 00	84.66± 5.03	157.99	38.00
64. *Chrysosporium tropicum*	GPCK 644	130.33± 10.59	141.00± 9.64	271.33	52.00
65. *Chrysosporium tropicum*	GPCK 645	196.66± 11.15	159.00± 5.00	355.66	64.00
66. *Chrysosporium tropicum*	GPCK 646	63.33± 5.68	96.66±14.15	159.99	13.00
67. *Chrysosporium tropicum*	GPCK 647	105.33± 10.21	101.00± 4.58	206.33	24.00
68. *Chrysosporium sulfurium*	GPCK 675	149.66± 43.93	186.66± 8.08	336.32	89.00
69. *Chrysosporium sulfurium*	GPCK 676	89.66± 16.50	135.33±13.31	224.99	71.00
70. *Chrysosporium zonatum*	ITCC 4732	127.66± 4.00	108.66± 5.13	236.32	40.00
71. *Chrysosporium zonatum*	GPCK 698	244.33± 3.00	179.66±10.01	423.99	36.00
72. *Ctenomyces serratus*	GPCK 677	115.33± 6.42	138.66±16.65	253.99	14.00
73. *Ctenomyces serratus*	GPCK 678	50.33± 16.56	77.33± 0.57	127.66	15.00
74. *Ctenomyces serratus*	GPCK 679	134.00± 0.57	107.00± 4.35	241.00	21.00
75. *Ctenomyces serratus*	P 131	180.00± 0.57	209.00± 9.00	389.00	66.00
76. *Gymnoascus petalosporus*	MTCC 2600	171.33± 51.63	221.00±44.57	392.33	59.00
77. *Malbranchea* sp.1	GPCK 680	143.00± 6.08	171.33± 4.50	314.33	62.00
78. *Malbranchea* sp.2	GPCK 681	96.66± 11.50	138.00±13.74	234.66	28.00
79. *Malbranchea* sp.3	GPCK 682	108.00±00	131.00±22.00	239.00	17.00
80. *Malbranchea* sp.4	GPCK 683	65.00± 5.00	108.66±10.59	173.66	15.00
81. *Malbranchea* sp.5	GPCK 684	49.00± 2.00	80.66± 2.08	129.66	12.00
82. *Malbranchea* sp.6	GPCK 685	104.00±00	125.33± 5.03	229.33	32.00
83. *Malbranchea* sp.7	GPCK 686	164.00± 1.00	134.00± 6.00	298.00	45.00
84. *Microsporum gypseum*	GPCK 687	104.66± 5.77	84.66± 2.51	189.32	71.00
85. *Microsporum gypseum*	GPCK 688	249.66± 11.06	97.66± 8.02	347.32	91.00
86. *Microsporum gypseum*	GPCK 689	207.66± 17.00	83.00± 3.05	290.66	90.00
87. *Microsporum gypseum*	GPCK 690	151.66± 11.50	130.33± 9.60	281.99	73.00
88. *Microsporum gypseum*	GPCK 691	68.33± 17.55	73.66± 0.57	141.99	27.00
89. *Microsporum gypseum*	GPCK 692	126.66± 45.36	77.00± 1.73	203.66	38.00
90. *Microsporum gypseum*	GPCK 693	251.33± 14.22	273.66± 8.14	524.99	69.00
91. *Microsporum gypseum*	GPCK 694	203.00± 8.18	119.33± 5.68	322.33	63.00
92. *Microsporum gypseum*	GPCK 695	213.00± 8.88	152.66± 2.08	365.66	82.00
93. *Microsporum gypseum*	GPCK 696	120.00± 0.57	126.33± 3.51	246.33	63.00
94. *Microsporum gypseum*	GPCK 697	200.00± 1.00	144.00± 4.00	344.00	81.00
95. *Microsporum gypseum*	ITCC 4733	345.33± 38.43	353.33±28.88	698.66	98.00
96. *Narashimella hyalinospora*	FMR 4086	39.33± 7.63	91.33± 3.51	130.66	13.00
97. *Narashimella marginospora*	FMR 4087	20.00± 7.81	81.00± 1.00	101.00	9.00
98. *Verticillium tenuipes*	FMR 6214	107.01± 2.52	107.66±14.01	214.66	65.00
99. *Verticillium tenuipes*	FMR 6215	121.66± 17.03	143.66± 3.21	265.32	64.00
100. *Verticillium tenuipes*	FMR 6216	58.33± 15.53	93.66± 2.51	151.99	18.00

a) Mean+S.D.=Significant at $P<0.05$, b) Total of mean, c) Calculated %.

250-ml Erlenmeyer flask received 200 mg of feathers, 100-ml of mineral medium, and fungal inoculum. Fungus controls and keratin controls were also run along with the test sample. The flasks were incubated in a stationary condition at 28±2°C in the dark.

The decomposition of feathers was assessed by the method of Kunert (1972). The protein released from feathers by the action of fungi was assayed by the methods of Lowry et al. (1951) and Nigam and Kushwaha (1992). Samples were taken from each flask after 30 d of incubation. The results of total values were represented as sum of mean of test sample, fungus and keratin controls (±S.D.) and weight loss was calculated as percent loss (Nigam and Kushwaha, 1992). All the experiments were carried out in triplicate at 28±2°C.

The protein released from the test flasks and weight loss of feathers are shown in Table 1. The highest protein release (683.66 and 698.66 μg/ml) occurred with *Aphanoascus fulvescens* (Cooke) Apinis GPCK 610 and *Microsporum gypseum* (Bodin) Guiart & Grigorakis ITCC 4733. *Microsporum gypseum* GPCK 693, *Botryotrichum piluliferum* Saccardo & Marchal GPCK 643, and *Acremonium* sp. GPCK 537 also released large amounts of protein. *Chrysosporium europae* Sigler, Guarro & Punsola, *Acremonium* sp. GPCK 538, *A. fulvescens* GPCK 641, *C. zonatum* Al-Musallam & Tan ITCC 4732, *Acremonium* sp. GPCK 539, and *C. keratinophilum* D. Frey ex Carmichael GPCK 658 showed protein release almost comparable to *Acremonium* sp. GPCK 537. Sufficient amount of protein was released by *C. keratinophilum* GPCK 661, *Gymnoascus petalosporus* Orr, Roy & Ghosh MTCC 2600, *Ctenomyces serratus* Ediam P 131, *C. tropicum* Carmichael GPCK 645, *C. indicum* (Randhawa & Sandhu) Garg GPCK 649, *C. queenslandicum* Apinis & Rees GPCK 668, *C. pannicola* (Corda) Van Oorschot & Stalpers GPCK 670, *C. sulfureum* (Fiedl) Van Oorschot and Samson GPCK 675 and *Malbranchea* sp. GPCK 680. *Acremonium* sp. GPCK 548, *N. marginospora* (Kuehn & Orr) Currah FMR 4068, *Acremonium* sp. GPCK 551, and *C. indicum* GPCK 639 released minimum protein. All other fungi tested here also released a small amount of protein. The results indicated that out of 100 fungi used, 2 were able to release above 600 μg/ml and 1 below 100 μg/ml protein (Fig. 1). The modal amount of protein released by the fungi tested was 200 μg/ml, and most of the strains were able to release between 300 and 400 μg/ml of protein (Fig. 2).

Maximum weight loss (98.0%) was caused by *M. gypseum* ITCC 4733. *A. fulvescens* GPCK 610, *A. fulvescens* GPCK 641, *M. gypseum* GPCK 688, *C. sulfureum* GPCK 675 and *C. europae* also showed high weight loss. *Chrysosporium indicum* GPCK 639, *Acremonium* sp. GPCK 548, *N. marginospora*, *C. indicum* GPCK 656, *C. keratinophilum* P 318 and *C. pannicola* GPCK 670 brought about minimum weight loss. All other test fungi also exhibited weight loss. Over 80% weight loss was shown by 9 fungi, more than 40 percent weight loss was achieved by 27 fungi, and remaining of the fungi caused 10 to 40% weight loss of feather (Fig. 3). The results of protein release and weight loss did not reveal any correlation, as shown by Nigam and Kushwaha (1992).

The results presented here clearly show that the fungi which grow on feathers released a high amount of protein in the medium, as indicated by Kushwaha (1995).

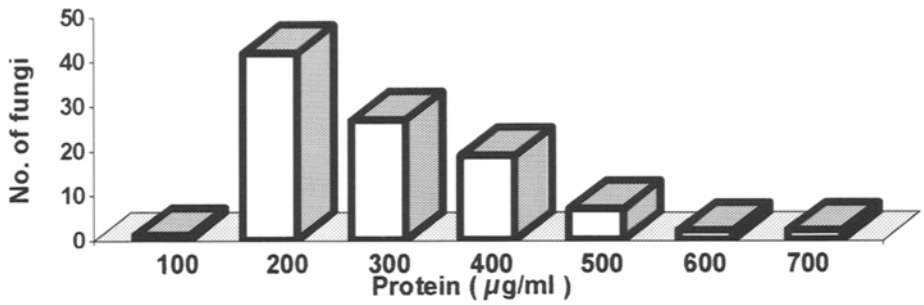

Fig. 1. Number of fungi showing protein released.

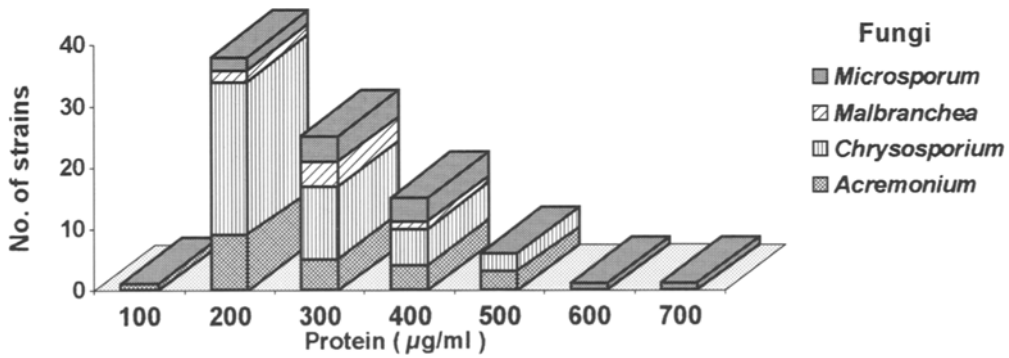

Fig. 2. Number of strains of four fungi showing protein released.

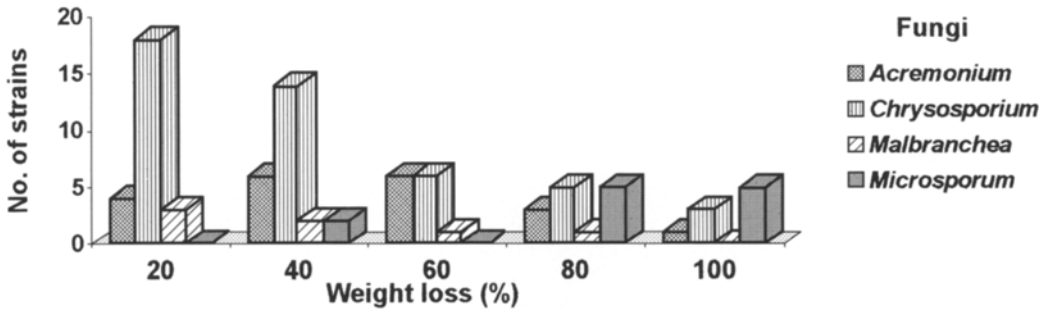

Fig. 3. Number of strains of four fungi showing weight loss.

Some strains which are of common occurrence in soil were found to degrade feathers and release a large amount of protein. It is possible, therefore, that these fungi may be used to utilize keratinized waste.

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Literature cited

Ainsworth, G. C. and Austwick, P. K. C. 1973. Fungal diseases of animals. Rev. Ser. **6**, CAB, England.

Ajello, L. 1960. Geogrphical distribution and prevalence of the dermatophytes. Ann. N. Y. Acad. Sci. **89**: 30–38.

Ajello, L. 1974. Natural history of the dermatophytes and related fungi. Mycopathol. Mycol. Appl. **53**: 93–110.

Alteras, I., Feuerman, E. J., Grunwald, M. and Shvili, D. 1984. *Tinea capitis* due to *Microsporum canis* in infants. Mycopathologia **86**: 89–92.

Currah, R. S. 1985. Taxonomy of the Onygenales: Arthrodermataceae, Gymnoascaceae, Myxotricaceae and Onygenaceae. Mycotaxon **24**: 1–216.

Dixit, A. K. and Kushwaha, R. K. S. 1990. Keratinophilic fungi of Andman Islands, India. Ind. J. Microbiol. **30**: 349–350.

Garg, A. P., Gandotra, S., Mukerji, K. G. and Pugh, G. J. F. 1985. Ecology of keratinophilic fungi. Proc. Ind. Acad. Sci. (Plant Sci.) **94**: 149–163.

Jain, M., Shukla, P. K. and Shrivastava, O. P. 1985. Keratinophilic fungi and dermatophytes in Lucknow soil and their global distribution. Mykosen **28**: 98–101.

Kunert, J. 1972. The digesion of human hair by the dermatophyte *Microsporum gypseum* in submerged culture. Mykosen **15**: 59–71.

Kushwaha, R. K. S. 1995. Biodeterioration of feathers by keratinomycetes isolated from a museum of Spain. 3rd International Conference on Biodeterioration of Cultural Property, Bangkok, Thailand, July 4–7, 1995, pp. 289–302.

Kushwaha, R. K. S. 1998. Biodegradation of keratin by soil inhabiting keratinophilic fungi, (ed. by Verma, A.), pp. 273–280. Malhotra Publishing House, New Delhi.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurements with folin phenol reagent. J. Bio. Chem. **193**: 265–275.

Mercantini, R., Marsella, R., Caprilli, F. and Dovgiallo, G. 1980. Isolation of dermatophytes and correlated species from the soil of public gardens and parks in Rome. Sabouraudia **18**: 123–128.

Nigam, N. and Kushwaha, R. K. S. 1992. Biodegradation of wool by *Chrysosporium keratinophilum* acting singly or in combination with other fungi. Trans. Mycol. Soc. Japan **33**: 481–486.

Nigam, N. and Kushwaha, R. K. S. 1993. Ecology of soil inhabiting keratinophilic fungi with special reference to *Chrysosporium*. pp. 173–182. Rastogi Publications, New Delhi.

Philpot, C. M. 1978. Geographical distribution of dermatophytes. A review. J. Hyg. **80**: 301–304.

Salebiau, A. and Carlas da Silva, L. 1980. Isolation of dermatophytes from hair of wild animals. An. Bras. Dermatol. **55**: 125–130.

Simordova, M. and Hejtmanek, M. 1970. Dermatophytes and other keratinophilic fungi in surface and waste water. Mykosen **13**: 467–471.

Sur, B. and Ghosh, G. R. 1980. Keratinophilic fungi from Orissa, India, I. Isolation from soils. Sabouraudia **18**: 269–274.