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Degradation of chicken feathers by *Chrysosporium georgiae*

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Abstract

Using a baiting technique, *Chrysosporium georgiae* was isolated from chicken feathers. Twenty-eight different fungal isolates were evaluated for their ability to produce keratinase enzymes using a keratin–salt agar medium containing either white chicken feathers or a prepared feather keratin suspension (KS). The *Chrysosporium* species were able to use keratin and grow at different rates. *Chrysosporium georgiae* completely degraded the added keratin after 9 days of incubation. Degradation of feathers by *C. georgiae* was affected by several cultural factors. Highest keratinolytic activity occurred after 3 weeks of incubation at 6 and 8 pH at 30 °C. *Chrysosporium georgiae* was able to degrade white chicken feathers, whereas bovine and human hair and sheep wool were not degraded and did not support fungal growth. Addition of 1% glucose to the medium containing keratin improved fungal growth and increased enzyme production. Higher keratin degradation resulted in high SH accumulation and the utilization of the carbohydrate carbon in the medium resulted in high keto-acid accumulation but decreased ammonia accumulation. Supplementation of the keratin–salt medium with minerals such as NH_4Cl and $MgSO_4$ slightly increased mycelial growth, but decreased production of extracelluar keratinase. Keratinase enzymes were very poorly produced in the absence of keratin, indicating its inducible nature. Analysis of endocellular keratinases in the mycelial homogenate indicated higher activity of intracellular keratinase as compared to the extracellular enzyme in culture filtrates. *Chrysosporium georgiae* was the most superior for keratinase production among the *Chrysosporium* species tested in the presence or absence of glucose. It produced more of the intracellular enzymes than the exocellular ones.

Key words: biochemical analysis, *Chrysosporium* spp., degradation, feathers

Introduction

Chicken feathers constitute a troublesome waste product that is produced in large quantities in commercial poultry processing plants and their utilization is of economic value as well as ecological significance to reduce pollution problems. Currently, feather waste is utilized on a limited basis as a dietery protein supplement for animal feedstuffs. Prior to its use for that purpose, feathers were chemically treated to increase their digestibility, a process that may have destroyed some of their amino acids. Therefore, enzymatic biodegradation may be a better alternative to improve their nutritional value.

The possibility of using keratinolytic microorganisms to increase the digestibility of feather waste has been studied by several authors. Wawrzkiewicz et al. [1] tested the ability of 16 strains of 12 species of dermatophytes to utilize keratin substrates (guinea pig hair and chicken feathers) and reported that guinea pig hair was degraded by *Trichophyton mentagrophytes, T. verrucosum* and *T. ajelloi*. Only *Microsporum gallinae* grew on native keratin from chicken feathers. Kushwaha [2] studied the degradation of peacock feathers by the enzymes of 20 different fungi and reported that some dermatophytes were active in this respect. Williams et al. [3] isolated a feather-degrading bacterium from a poultry waste digestor and adapted it to grow on feathers as its source of carbon, sulfur and energy. The organism was identified as *Bacillus licheniformes* and was reported to produce an extracellular keratinase [4]. Further search for microorganisms with a special affinity for chicken feathers is still needed.

Studies on conditions controlling the feather degradation process have been very few and the mecha-

nism is still debatable. In this study we report on chicken feather degradation by some fungal species of the genus *Chrysosporium* with special reference to *C. georgiae*.

Materials and methods

Micro-organisms

The fungi used in this study were obtained from the following sources: isolates numbered 6 to 21 are listed in Table 1. They were obtained from the Mycological Laboratory, Botany Department, Faculty of Science, Assiut University, while isolates 22 to 41 were provided by the Botany Department, Faculty of Science at Sohag, South Vally University. Isolates 1–5 (*C. georgiae, C. indicum, C. keratinophilum, C. pannicola* and *C. tropicum*), were isolated from chicken feathers collected from different localities in the El-Minia Governorate by the baiting technique described by Ajello [5], accordingly, feather samples were plated on the surface of double sterilized soil. The soil was moistened with sterile distilled water and remoistened whenever necessary. The plates were incubated at 37 °C for 10 weeks and the moulds that appeared on a feather's surface were transferred to Sabouraud's dextrose agar medium [6] and incubated at 30 °C for 10 days. The isolates were identified according to Carmichael [7] and kept on Sabouraud's medium at 4 °C for further studies.

Preparation of keratin substrate

Feathers. White chicken feathers were washed with warm water and a detergent, followed by defatting and then surface sterilized with petroleum–ether over 24 h, washed with glass distilled water and air dried. Portions of the substrate were then sterilized in separate vials by tindallization (80 °C for 30 min on three successive days with intervening incubation at 30 °C) according to Rajak et al. [8].

Soluble feather keratin preparation (SFKP). A 10 g sample of chicken feathers was heated in a reflux condenser at 100 °C for 2 h with 500 ml dimethylsulphoxide (DMSO). The DMSO-extracted keratin contained 1.2% protein. The mixture was placed in a refrigerator for 2 h to allow maximum precipitation of the keratin. After which it was centrifuged at $\times 6000$ *g* and the sediment was washed with four vol of water per vol of precipitate. The precipitate was then suspended in 0.1 M phosphate buffer at pH 8. This keratin suspension (KS) was used for further experiments at a final concentration of 0.06% protein.

Screening of fungal keratinolytic activities

The keratinolytic properties of the tested fungi were examined on a solid mineral medium containing 1.5% agar (Difco), or in a liquid medium in sterile Erlenmeyer flasks (100 ml) containing 20 ml of autoclaved basal salts medium containing (g/l): K_2HPO_4-1.5; $MgSO_4 \cdot 7H_2O$-0.025; $CaCl_2$-0.025; $FeSO_4 \cdot 7H_2O$-0.015 and $ZnSO_4$. $7H_2O$-0.005 and amended with feathers, 50 mg/flask or 0.06% KS, with or without 1% glucose in 1 l distilled water. The medium was adjusted to pH 7 with 0.1 N HCl, and the flasks inoculated with a spore suspension obtained from ten-day-old cultures of the test fungus and incubated for 21 days at 30 °C. Two replicates were used for each treatment.

Effect of incubation time, pH and incubation temperature

Only *C. georgiae* was used. It was grown on the basal salt solution containing 0.25% defatted white chicken feathers as the sole C and N sources at an initial pH 7. Inoculated flasks were incubated at 37 °C and keratinolytic activity was determined at 3-day intervals during an incubation period of 30 days.

The effect of different glucose concentrations (0, 0.1, 0.5, 1, 2 and 5%) on the production of keratinase by *C. georgiae* was tested in the basal salt solution containing 0.25% defatted white chicken feathers at an initial pH 7 and 30 °C for 21 days.

For studying the effect of incubation temperature, *C. georgiae* was grown for 21 days on the basal salt solution containing 0.25% defatted white chicken feathers at an initial pH 7 and different temperatures ranging from 15–45 °C. At the end of the incubation periods, final pH, dry weight, proteins and keratinase activity were determined. The effect of the initial pH of the medium was tested at 30 °C and different pH values ranging from 3–9.

Degradation of different keratin substrates

Chrysosporium georgiae was grown for 21 days on the basal medium containing 0.25% defatted keratin from chicken feathers, cow hair, duck feathers, sheep wool, rabbit hair, human hair and KS as C and N sources in the presence or absence of 1% glucose at an initial

Table 1. Growth and keratinase activity[a] of some fungal species on white chicken feathers and a keratin suspension (Ks) after 21 days at 30 °C and pH 7.

No.	Fungal species	Fungal growth on chicken feathers (weeks)			Diameter of fungal colonies and enzyme activity on KS (mm)			
		First week	Second week	Third week	3 days	6 days	9 days	12 days
1.	*Chrysosporium pannicola*[d]	+	++	+++	18[b] (19)[c]	42 (43)	73 (74)	95 (96)
2.	*C. keratinophilum*[d]	+	++	+++	23 (25)	44 (46)	69 (71)	88 (90)
3.	*C. tropicum*[d]	+	++	+++	22 (26)	31 (31)	43 (43)	47 (47)
4.	*C. indicum*[d]	+	++	+++	24 (26)	37 (40)	43 (44)	49 (50)
5.	*C. georgiae*[d]	+	++	+++	35 (37)	75 (77)	92 (94)	D
6.	*Microsporum gypseum*	–	+	++++	–	–	–	4 (6)
7.	*M. boullardii*	–	–	+	–	–	6 (6)	10 (10)
8.	*M. racemosum*	–	+	+	–	–	5 (8)	9 (11)
9.	*Trichophyton mentagrophytes*	–	+	+	5 (7)	20 (22)	29 (31)	42 (45)
10.	*T. rubrum*	–	+	+	–	2 (5)	6 (8)	10 (12)
11.	*T. terrestre*	–	+	+	–	–	8 (10)	14 (17)
12.	*Aspergillus flavus*	+	+	+	14 (–)	36 (–)	48 (–)	61 (–)
13.	*A. fumigatus*	+	+	+	17 (–)	33 (–)	46 (–)	63 (–)
14.	*A. terreus*	+	+	+	12 (–)	17 (–)	25 (–)	41 (–)
15.	*A. sydowi*	–	–	+	6(-)	11 (–)	19 (–)	28 (–)
16.	*A. nidulans*	–	–	+	–	4 (–)	12 (–)	20 (–)
17.	*A. ustus*	+	+	+	23 (–)	41 (–)	67 (–)	89 (–)
18.	*A. niger*	+	+	+	27 (–)	56 (–)	71 (–)	93 (–)
19.	*Penicillium chrysogenum*	–	+	+	–	7 (–)	12 (–)	23 (–)
20.	*P. funiculosum*	–	+	+	4 (–)	11 (–)	19 (–)	28 (–)
21.	*P. jensenii*	–	–	+	–	2 (–)	6 (–)	7 (–)
22.	*Chrysosporium tropicum* (1)	+++	+++	+++	11.2 (11.2)	34 (34)	48.2 (48.2)	
23.	*C. tropicum* (2)	+++	+++	+++	12.2 (12.2)	32.5 (32.5)	50.5 (50.5)	
24.	*C. rubrum* (1)	–	+	+	12.5 (13.2)	23.7 (27.2)	34.7 (38.7)	
25.	*C. rubrum* (2)	–	+	+	16.5 (18)	30.2 (33)	39.5 (42.2)	
26.	*C. indicum* (1)	+	+++	++++	15.7 (15.7)	34.5 (34.5)	48.2 (48.2)	
27.	*C. indicum* (2)	+++	++++	++++	16 (16.2)	38.2 (38.2)	54.2 (54.2)	
28.	*C. keratinophilum* (1)	+++	++++	++++	7 (–)	10 (10)	21.2 (21.2)	
29.	*C. keratinophilum* (2)	+++	++++	++++	14.2 (14.2)	33 (33)	49.7 (49.7)	
30.	*C. xerophilum* (1)	++	++	+++	21.5 (21.5)	36 (36)	47 (47)	
31.	*C. xerophilum* (2)	++	++	+++	13 (13)	36.2 (36.2)	48.7 (48.7)	
32.	*Trichophyton equinum* (1)	+	+++	++++	8.7 (8.7)	30 (30)	40 (40)	
33.	*T. equinum* (2)	+	+++	+++	16.5 (19.5)	29.2 (29.2)	39.7 (39.7)	
34.	*Chrysosporium asperatum* (1)	++	+++	+++	10.2 (4)	21.7 (21.7)	33 (33)	
35.	*C. asperatum* (2)	+	++	+++	5 (4)	18.2 (18.2)	28.7 (28.7)	
36.	*C. luteum* (1)	+++	++++	++++	11.2 (11.2)	36.5 (36.5)	48.7 (48.7)	
37.	*C. luteum* (2)	+++	+++	++++	14 (14)	34.5 (34.5)	50.2 (50.2)	
38.	*C. lucknowense* (1)	+	+++	+++	6.5 (6.5)	21.2 (21.2)	31.2 (31.2)	
39.	*C. lucknowense* (2)	++	+++	++++	15 (15)	29.2 (29.2)	38.7 (38.7)	
40.	*Arthroderma cuniculi*	+	+	++	12.5 (12.5)	25 (26)	33 (34.2)	
41.	*Chrysosporium queenslandicum*	++++	++++	++++	15 (15)	36.7 (36.7)	50.2 (50.2)	

[a] Visual estimations.
[b] Fungal colony diameter.
[c] Zone diameter of keratin solubilization.
[d] Isolated from chicken feathers.
–, no fungal growth; +, low fungal growth; ++, moderate fungal growth; +++, good fungal growth; ++++, high fungal growth.
D, dissolution of keratin protein suspension (Ks).

pH of 7 and an incubation temperature of 30 °C. At the end of the incubation period, final pH, growth, weight, degradation products and keratinase activity were determined.

Effect of C, N, and S on feather degradation

Chrysosporium georgiae was grown for 21 days on the basal medium supplemented with 0.25% defatted white feathers to which were added 1% glucose, 30 mM NH_4Cl and 0.05% $MgSO_4$ in one, two, three or four combinations at an initial pH of 7 and incubation temperature of 30 °C. At the end of the incubation period, final pH, growth, degradation products and keratinase activity in the culture filtrates and mycelium homogenates were determined.

Biochemical analysis

The protein content of the culture filtrate of *C. georgiae* was determined according to Lowery et al. [9], sulfhydril content by the method of Ellman [10], ammonia was determined colorimetrically by the method developed by Delory [11] and modified by Naguib [12], keto-acids by the method of Freidman and Hauge [13], amino acids by a method similar to that of Muting and Kaiser [14] and keratinase activity by a method similar to that of Tomarelli et al. [15]. Dry weight was determined by drying for 24 h at 80 °C to constant weight.

Results

Degradation of chicken feathers and keratinase production by 28 different fungal isolates, some of them dermatophytes, were evaluated using keratin–salt agar media containing either white chicken feathers or KS. Fungal growth was observed either visually or determined by measuring colony diameters, at intervals during the incubation period which lasted for three weeks. The production of extracellular keratinase was also evaluated by measuring the diameters of the clear zones around the fungal colonies.

Table 1 shows that among the fungi tested the *Chrysosporium* species were able to use keratin and grow with different magnitudes of development. *Chrysosporium indicum, C. keratinophilum, C. tropicum, C. queenslandicum* and *C. georgiae* were the superior ones. The last fungal species completely disintegrated the added keratin after 9 days of incubation and grew luxuriantly; therefore it was selected for further experimentation. Growth of the *Microsporum* species on feathers and KS was poor, whereas the *Trichophyton* species used KS much better than feathers and *T. rubrum* and *T. equinum* were the most superior. The different *Aspergillus* species tested (*A. ustus, A. niger* and *A. fumigatus*), although they grew luxuriantly on KS they grew very poorly and did not show any extracellular keratinase activity. The *Aspergillus* spp. colonies did not exhibit any keratinolytic activity. The differences in growth responses may indicate some degradation of keratin in KS, although both KS and native keratin are chemically similar as indicated by their infrared spectra (Figure 1).

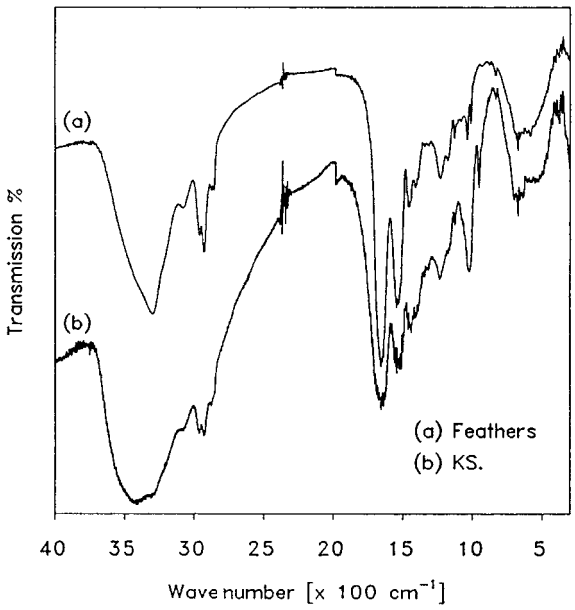

Figure 1. Infrared spectra of chicken feather and a keratin suspension (KS).

Testing the effect of some cultural factors on feather degradation and production of keratinase in the liquid feather–salts medium by *C. georgiae* revealed that the highest keratinolytic activity occurred after three weeks of incubation at 37 °C and pH 7. Also pHs 6.0 and 8.0 were the most favourable for keratinase production. Testing the effect of incubation temperatures on keratinase production at pH 8.0 revealed that incubation at 30 °C was most favourable for enzyme production.

Degradation of feather keratin by *C. georgiae* was compared with degradation of other keratin sources. The results in Table 2 show that chicken feather keratin was the most active for degradation by *C. georgiae* enzymes under the conditions of the exper-

iment; whereas cow hair, human hair and sheep wool were not degraded and did not support fungal growth. However, the addition of 1% glucose to the medium, containing any of the keratin sources tested, improved fungal growth and increased enzyme production. Chicken feathers were the most favorable for keratinase production (Table 3). Supplementation of the chicken feather keratin–salt medium with 1% glucose almost doubled fungal growth as well as extracellular keratinase production. Higher keratin degradation resulted in high SH accumulation and the utilization of carbohydrate carbon in the medium resulted in high keto-acid accumulations but decreased ammonia accumulation. The addition of NH_4Cl to the medium together with glucose, although it increased fungal growth, decreased keratinase production, accumulation of SH groups, soluble proteins and keto-acids but increased accumulation of ammonia. Supplementation of the keratin–salt medium with NH_4Cl only slightly increased mycelial growth, but drastically decreased keratinase production and SH accumulation. Supplementation of the keratin–salt medium with $MgSO_4$, although it slightly increased mycelial growth, decreased production of extracellular keratinase in the culture filtrate as well as the accumulation of SH groups. In the presence of 1% glucose, the addition of $MgSO_4$ did not improve mycelial growth, but decreased keratinase production. Addition of $MgSO_4$ to the keratin–salt medium containing NH_4Cl did not improve mycelial growth, but increased keratinase production. Addition of glucose to this medium increased mycelial growth, but did not improve keratinase production. Keratinase was very poorly produced in the absence of keratin, thus indicating its inducible nature. Replacing keratin by glucose at 1% concentration resulted in luxuriant mycelial growth but almost inhibited keratinase production.

Analysis of endocellular keratinase in the mycelial homogenate indicated higher activity of intracellular keratinase as compared to the extracellular enzyme determined in the culture filtrate. Production of the intracellular enzyme was particularly stimulated by the addition of glucose to the keratin–salt medium (Table 4).

Since keratinase production and degradation of chicken feathers by *C. georgiae* were improved by addition of glucose, it was of interest to determine the optimum glucose concentration that would stimulate enzyme production and substrate degradation. Table 5 shows that the most favorable glucose concentration ranged between 0.1–1% with the high concentration being more favorable for mycelial growth, but it decreased ammonia production and the pH of the medium.

Under the most favorable conditions for keratinase production (extra and intracellular), it was of interest to compare the production of keratinase enzymes by the *Chrysosporium* species that were determined in this study to be the most keratinolytic, namely, *C. indicum, C. keratinophilum, C. luteum, C. queenslandicum* and *C. xerophilum* with that of *C. georgiae*. Results in Table 6 shows that *C. georgiae* was superior for keratinase production in the presence or in the absence of glucose; it produced more intracellular enzyme than the exocellular one.

Chrysosporium keratinophilum produced amounts of intracellular enzyme of similar magnitude to that produced by *C. georgiae*, but it produced less extracellular enzyme. *Chrysosporium queenslandicum* was similar except that the production of these enzymes was not highly stimulated by glucose. In the presence of glucose, both *C. xerophilum* and *C. luteum* produced the highest amounts of extracellular keratinase. *Chrysosporium indicum*, on the other hand, produced the least extra and intracellular enzymes both in the presence and in the absence of glucose.

Discussion

Experiments on the degradation of chicken feathers by 28 different fungal species indicated that fungi belonging to the genus *Chrysosporium* were the most active in degrading feathers and that *C. georgiae* was the most active one. Keratin solubilized in DMSO, although chemically similar to native keratin, produced better mycelial development and could support good growth of several species that grew very poorly on feathers. This suggested that the native keratin of feathers did not represent as good a source of C and N for these fungi as the KS preparation, and that the KS preparation was only partially degraded. A similar finding was also reported by Wawrzkiewicz et al. [1, 16] during their work on feather degradation by *M. gallinae*. Optimum degradation of feather keratin by *C. georgiae* was obtained after 21 days at pH 6 or 8 and an optimum incubation temperature of 30 °C. Wawrzkiewicz et al. [17] reported that *M. gallinae*'s enzymes that degraded feather keratin were more active at pH 8 than at pH 6. Similar results were also reported for *T. rubrum*'s enzymes that degraded wool [18, 19], hair, horn [20] and human hair keratins [21].

Table 2. Effect of different keratinaceous substrates on the growth and enzymatic activity of *Chrysosporium georgiae* in saline medium at 30 °C, pH 8 and an incubation period of 21 days

Substrates	Growth Sporulation	F Wt (g/Flask)	Final pH	Sol protein (mg/ml)	Ammonia (μg/ml)	Keto acids (μg/ml)	Amino acids (μmol/ml)	Keratinase activity (U/Flask)
Chicken feathers	+	0.527	7.31	0.459	8.05	2.85	16.76	135
Cow hair					No fungal growth			
Duck feathers	±[a]	0.406	7.10	0.534	6.93	1.29	27.07	104
Sheep wool					No fungal growth			
Rabbit hair	+	0.449	7.10	0.194	5.45	1.02	36.10	62.5
Human hair					No fungal growth			
(KS)	++	0.812	7.31	0.257	5.28	1.76	59.31	70

+: low fungal sporulation, ++: moderate fungal sporulation; [a] weak fungal growth with no sporulation.

Table 3. Effect of different keratinaceous substrates on the growth and enzymatic activity of *Chrysosporium georgiae* in saline medium containing 1% at 30 °C, pH 8 and an incubation period of 21 days

Substrates	Growth Sporulation	F Wt (g/Flask)	Final pH	Sol protein (mg/ml)	Ammonia (μg/ml)	Keto acids (μg/ml)	Amino acids (μmol/ml)	Keratinase activity (U/Flask)
Chicken feathers	+++	0.965	4.20	5.21	0.000	15.61	612.4	220.0
Cow hair	++[a]	0.615	4.17	3.33	0.303	13.91	524.7	110.0
Duck feathers	++	0.962	4.19	4.23	1.730	11.74	193.4	175.0
Sheep wool	+++[b]	0.833	3.94	4.01	0.000	13.17	878.7	102.5
Rabbit hair	+++	1.267	4.10	4.89	0.173	14.12	960.6	72.5
Human hair	++[c]	1.416	4.06	2.82	0.563	15.21	473.2	132.0
(KS)	+++[d]	0.415	4.30	6.63	0.779	17.85	899.9	125.0

++: moderate fungal sporulation; +++: good fungal sporulation.
[a] Growth surrounds hairs and hairs turn brown.
[b] Growth surrounds wool and wool turns brown.
[c] Growth surrounds hairs.
[d] Total dissolution of KS with clear brown filtrate.

Table 4. Keratin degradation by *Chrysosporium georginae* in the presence of keratin and different combinations of glucose (1%), NH_4Cl (30 mM) and $MgSO_4 \cdot 7H_2O$ (0.05%) at 30 °C at pH 8.0 for 21 days

Treatment	Growth		Final	Sol protein	SH group	Keratinase activity		Ammonia	Keto acids	Amino acids
	Sporulation	F wt (g/f)	pH	(mg/ml)	(mmol/f)	Filtrate (u/f)	Homogenate (u/f)	(μg/ml)	(μg/ml)	(μg/ml)
Feathers	+	0.450	7.06	0.843	0.123	45	16.75	16.94	2.83	174.42
Feathers + Glucose	+++	0.899	5.29	4.688	0.639	105	728.25	10.56	48.39	600.00
Feathers + NH_4Cl	++	0.555	6.21	1.091	0.031	19.5	1.875	47.41	3.51	385.43
Feathers + $MgSO_4$	+	0.604	7.19	0.864	0.071	21.11	79.15	17.60	3.02	537.43
Feathers + Glucose + NH_4Cl	++++	1.020	5.43	2.229	0.111	73.33	274.95	30.36	6.91	800.00
Feathers + Glucose + $MgSO_4$	++++	0.864	5.01	0.971	0.537	112.22	420.82	15.07	42.15	604.14
Feathers + NH_4Cl + $MgSO_4$	+	0.596	6.22	2.119	0.039	30	112.5	34.76	2.00	834.00
Feathers + Glucose + NH_4Cl + $MgSO_4$	++++	0.823	5.53	2.119	0.427	13.33	49.95	26.84	3.72	847.43
Glucose + NH_4Cl + $MgSO_4$	++++	0.727	4.63	4.748	0.018	8.88	33.3	32.23	4.61	1040.71

+: low fungal sporulation; ++: moderate fungal sporulation; +++: good fungal sporulation; ++++: high fungal sporulation.

Table 5. Efect of addition of different concentratons of glucose on growth and enzyme activity of *Chrysosporium georgiae* on chicken feathers in saline medium at 30 °C, pH 8 and incubation period of 21 days

Glucose (%)	Growth Sporulation	F Wt (g/Flask)	Final pH	Sol protein (mg/ml)	Ammonia (μg/ml)	Keto acids (μg/ml)	Amino acids (μmol/ml)	Keratinase activity (U/Flask)
0.0	+	0.406	7.31	0.459	8.05	2.85	16.76	135
0.1	+[a]	0.459	7.17	0.792	7.49	5.79	305.60	280
0.5	++[b]	0.847	6.36	3.446	1.43	30.07	447.40	246
1.0	+++[c]	0.965	4.20	5.214	0.00	15.61	612.40	220
2.0	+++[d]	1.010	4.29	6.245	0.00	13.44	1012.10	156
5.0	++[e]	0.086	4.18	8.528	0.173	4.48	123.8	81

+: low fungal sporulation; ++: moderate fungal sporulation; +++: good fungal sporulation.
[a] White sporulation and white filtrate.
[b] Pale brown sporulation and brownish filtrate.
[c,d] Dull yellow sporulation and yellowish filtrate.
[e] Brownish yellow sporulation and yellowish filtrate.

Table 6. Comparison of growth and keratinolytic activities of 6 different species of *Chrysosporium* on chicken feathers in a liquid mineral solution at pH 8 and an incubation period of 27 days

Species	Fresh weight (g/flask)		Final pH		Sol protein (mg/ml)		Keratinase activity			
							Filtrate (U/flask)		Homogenate (U/flask)	
	a	b	a	b	a	b	a	b	a	b
Chrysosporium georgiae	0.292	0.337	6.57	6.31	0.37	2.49	437.5	752.5	391	1750
Chrysosporium keratinophilum	0.295	0.334	6.68	6.43	1.62	9.42	280	482.5	448	1785
Chrysosporium indicum	0.327	0.466	6.62	6.39	1.14	3.36	53.7	103.7	276	416
Chrysosporium xerophilum	0.251	0.386	6.55	6.39	0.66	10.08	98.7	972.5	382	492
Chrysosporium luteum	0.403	0.395	6.43	6.26	1.21	11.48	170	1960	232	750
Chrysosporium queenslandicum	0.349	0.479	6.50	6.24	1.67	9.86	322.5	540	442	674

[a] Medium without glucose.
[b] Medium with 0.5% glucose.

A pH of 8.0 was also found to be optimum for feather degradation by a *Streptomyces fradiae* culture filtrate by Young and Smith [22]. The optimum pH for feather degradation by *Bacillus licheniformis* was also found to be 7.5 [3]. In the previous cases, the high optimum temperature of 50 °C was reported for enzyme activity. Our results indicated that optimum enzyme production by *C. georgiae* coincided with the optimum growth temperature of 30 °C. Under this condition, native chicken feathers constituted a good source of C and N for *C. georgiae* as they supported good growth within three weeks.

Feather degradation and production of keratinase enzymes on native feather medium were increased by the addition of 1% glucose; however, supplementation of the keratin medium with ammonium chloride was highly depressive to enzyme production while the addition of sulfate had only a slight effect. Such results were in accordance with the previous findings of Malviya et al. [23] for *C. queenslandicum*. They noted the keratinase enzyme was inducible by keratin and its production was stimulated by glucose and inhibited by ammonia.

The keratinase enzyme produced by *C. georgiae* and the other *Chrysosporium* species tested seems to be associated with the mycelium since more keratinase activity was detected in the mycelial homogenate than that detected in the culture filtrate. Production of only intercellular keratinase without any keratinolytic activity in the culture filtrate was reported for *M. gallinae* by Wawrzkiewicz et al. [17] on chicken feathers; on the other hand, production of extracellular keratinase during the growth of several keratinolytic fungi on natural keratin substrate is well established [24–26]. Wawrzkiewicz et al. [1] tested 16 strains of dermatophytes for their ability to utilize keratin substrates and reported that the strains produced mainly intracellular keratinases while only 3 strains of *T. verrucosum*

released enzymes into the medium. Production of extracellular enzymes by dermatophytes plays a significant role in allowing those organisms to grow and colonize insoluble host substrates. The production of intracellular enzymes located at or near the cell surface however would degrade keratin in the vicinity of the cell while extracellular enzymes would catalyze hydrolysis of keratin both close to and far from the mycelium. Yu et al. [24–26] isolated and purified both extracellular and cell bound keratinases from *T. mentagrophytes*. Our results are, however, in accordance with the opinion that two different enzymes are closely linked with the mechanism of keratinolysis by *C. georgiae* and that they probably act in conjunction with other mechanisms to degrade keratin such as mechanical penetration of the mycelium. Further work on this problem is continuing in our laboratory.

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