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Studies on the decomposition of keratin waste by saprotrophic microfungi. III. Activity and properties of keratinolytic enzymes

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It has been proved that saprotrophic keratinolytic fungi produce keratinolytic extra- and intracellular proteases during the process of decomposition of crude keratin waste (feathers). Some properties of these enzymes were studied (Korniłłowicz-Kowalska 1997a, 1977b).

Key words: decomposition of keratin, saprotrophic micromycetes, keratinolytic enzymes.

INTRODUCTION

In literature there are a lot of studies on the activity and properties of keratinolytic proteases of pathogenic fungi that cause skin mycosis in humans and animals (Meevootison and Niederpruem 1979; Takiuchi, Higuchi, Sei and Koga 1982, 1983; Takiuchi, Sei, Togaki and Negi 1984; Sanyal, Das and Banerjee 1985; Asahi, Lindquist, Fukuyama, Apodaca, Epstein and Mc Kerrow 1985; Tsuboi, Matsuda and Ogawa 1987; Wawrzkiewicz, Łobarzewski and Wolski 1987; Apodaca and Mc Kerrow 1989a, 1989b; Grzywnowicz, Łobarzewski, Wawrzkiewicz and Wolski 1989; Wawrzkiewicz, Wolski and Łobarzewski 1991; Qin, Dekio and Jidoi 1992 and others). From the above mentioned studies follows, among others, that pathogenic dermatophytes produce keratinolytic proteases, called keratinases. Studies by Yu, Ragot and Blank (1972) carried out on the cultures of *Trichophyton mentagrophytes* in the mineral-keratin medium showed the

presence of these enzymes both in the medium and mycelium. Extension of these studies on other species of zoo- and antropophilic dermatophytes (Meevootison and Niederpruem 1979; Takiuchi et al. 1982, 1983, 1984; Asahi et al. 1985; Apodaca and Mc Kerrow 1989; Grzywnowicz et al. 1989; Qin et al. 1992) showed that in the that fungi group production of extracellular keratinolytic enzymes is a widely spread feature. Moreover, keratinases of some dermatophytes are not released into the medium but deposited in the surface fungi structures as it was shown by the studies by Wawrzkiewicz et al. (1987); Łobarzewski, Grzywnowicz, Wawrzkiewicz and Staszczak (1990). Contrary to the pathogenic micromycetes studies on the keratinolytic proteases of saprotrophic microfungi are scarce (Chesters and Mathison 1963; Ragot 1966; Böhme 1967; Ragot, Benedicto and Tallieu-Calvert 1973; Kushwaha 1983). They do not present any information on the activities and properties of these enzymes.

Taking into consideration the fact that keratins are also part of the waste masses, studies on the proteases of saprotrophic keratinomycetes are not only of great importance from the ecological point of view, but can also allow for finding new directions in the utilization and neutralization of keratin waste.

The aim of the present study was to obtain basic information on the localization, activity, and some important biotechnological properties of the proteolytic enzymes that take part in the hydrolysis of native feather keratin.

MATERIAL AND METHODS

Fungi strains that were able to carry out complete solubilization of native feather keratin in the shortest possible time (i.e. in 70 days) were chosen for the present experiment (Korniłłowicz-Kowalska 1997). These were the following strains: *Arthroderma quadrifidum* (strain 1), *A. curreyi* (strain 2), and *Chrysosporium pruinosum* (strain 1).

Cultures were grown on the medium containing native feathers as the only source of C and N (medium II – Korniłłowicz-Kowalska 1997a) for 21 days (the phase of quick lysis). Enzymatic analyses were carried out in the cells free after-culture filtrates and fungi homogenates as well as their high molecular fractions, after 7, 14, and 21 days.

In order to obtain homogenates substrate, separated from after-cultrue fluids, together with mycelium was washed twice with the saline buffered to pH 7.8 and centrifuged. Fresh mass collected from 3–5 repetitions was weighed, and then samples of equal weight (about 30 g) were suspended in 28 mM buffer Tris-HCl with pH 7.8 in the ration 1:3. The prepared suspension was homogenized when cold for 10 min at 1400 rev · min^{-1} in a glass homogenizer.

Degree of desintegration of mycelium was evaluated by means of a microscope. Thick, homogeneous suspension was separated into 3 equal parts. Each part was then centrifuged for 15 min at 6000 rpm. Clear supernatant was treated as the source of intercellular enzymes (H_1). Centrifuged deposit was washed with the buffered saline two times and centrifuged each time. After washing deposit was weighed, and rubbed in a mortar using 5 g of etched river sand, 1 cm^3 28 mM of buffer Tris-HCl with pH 7.8 for 1 g of biological material and then rubbed again for 15 min. Then 9 cm^3 of buffer was added and it was rubbed again for 5 min. The suspension was centrifuged for 15 min at 6000 rpm. Deposit was rejected and supernatant was treated as a source of enzymes localized in the surface mycelium structures and marked as H_2. Clear after-culture filtrates (P) and homogenats (H) were stored for 24 hours at +4°C. Before they were used protein content was determined by Lowry method (Lowry, Rosenberg, Farr and Randall 1951). One cm^3 was taken for determinations of enzymatic fractions.

High molecular fractions (crude proteases preparations) were obtained by concentration and ultrafiltration of cell free after-culture filtrates and mycelium homogenats in the Amicon apparatus typ TCF 2A using Diaflo YM10 membranes and nitrogen atmosphere. Ultrafiltrates containing compounds with the mass of $< 10\,000$ daltons were rejected, whereas the remaining concentrate (2 cm^3) of the substance with molecular weight of $\geqslant 10\,000$ daltons was suspended in 28 mM phosphate buffer or Tris-HCl with pH of 7.8. The enzymatic material was cooled down by storing it for 24–48 hours at +4°C. Before usage protein content was determined by means of the methods described above.

Activity of keratinases in after-culture filtrates, mycelium homogenats, and macromolecular fractions (after Amicon) was determined in relation to chemically pure keratin as a substrate using method Takiuchi (Takiuchiu et al. 1982) adapted to the present study subject (crude filtrate, homogenat or 50 μg of protein in the case of crude enzymatic preparations instead of the solution of pure keratin and shortening of the incubation time).

Eight protein substrates, 3 globular proteins, and 5 fibrous proteins, with native chicken feather keratin and guinea pig hair included, were used for the studies of substrate spectrum of crude proteases preparations.

For the determinations of activity of enzymatic preparations in relation to globular protein 1% of casein solution ("Sigma"), albumine (BDH) and hemoglobin ("Sigma") were used. Enzymatic reactions were studied by the method used before (Korniłłowicz 1994; Korniłłowicz-Kowalska 1997a) using 1 cm^3 of crude enzyme preparations. The amount of TCA soluble products was measured on the basis of absorbance measurements at the wave length of 280 nm, calculating the values obtained as the units of enzymatic activity (U) with the assumption (Łobarzewski

et al. 1990) that 1 unit of enzymatic activity equals the amount of enzyme that in the standard conditions (37°C, pH 7.8) causes absorbance increase by 0.01 cm^{-3} min^{-1}. The final results were given in the units of mg^{-1} protein enzymatic activity.

Azocollitic activity was measured using 10 mg azocoll ("Sigma") in 1 cm^3 28 mM buffer Tris-HCl (+ 1 mM Mg^{2+}) with pH 7.8 and 1 cm^3 of enzymatic material in the same buffer. Enzymatic reaction was carried out at 37°C for 1 hour. In the control samples, enzyme was inactivated by boiling it for 30 min at the temperature of 100°C. After incubation the mixture was centrifuged for 5 min at 3000 rpm^{-1}, and absorbance was measured in the supernatant at the wave length of 520 nm assuming as 1 azocollitic unit (U) the amount of enzyme that causes absorbance increase by 0.100 cm^{-3} h^{-1} (A p o d a c a and Mc K e r r o w 1989a). Final results were expressed as described above.

Enzymatic activity of enzymes in relation to elastine ("Sigma") was determined using 10 mg of substrate suspended in 1 cm^3 of 28 mM buffer. The reaction mixture was incubated at 37°C for 3 hours, then it was centrifuged for 5 min at 3000 rpm^{-1}. In the control, the enzyme was inactivated for 30 min at 100°C. In the clear supernatant absorbance was measured at 280 nm. The values obtained were re-calculated in the enzymatic units (U) mg^{-1} of protein. The amount of enzyme that releases 1 mg of dissolved protein in h^{-1}(at 37°C, pH 7.8) was assumed as a elastinolytic unit (G a l a s and K a ł u ż e w s k a 1989). Activity of crude enzymatic preparations in relation to native keratin was determined using chicken feathers and guinea pig hair as substrates. Determinations were carried out in an identical way as for determinations of keratinolytic activity of non-fractioned after-culture filtrates described in the first part of the present study, prolonging incubation time up to 24 hours (natrium azide as stabilizer). The results obtained were expressed as before, measuring the amount of soluble protein at 280 nm.

Enzymatic reaction in relation to azokeratin (keratin azure manufactures by "Sigma") was carried out in the same way as for native feathers and hair. Measurements of absorbance were carried out at the wave length of 595 nm. The values obtained were recalculated for the units (U) mg^{-1} of protein according to the assumption (A p o d a c a and Mc K e r r o w 1989a) that 1 unit of enzymatic activity equals absorbance increase (A_{595}) by 0.01.

Determination of the pH influence on the activity of crude protease preparations of fungi were carried out in the 28 mM phosphate buffer (+ 1 mM Mg^{+2}) with pH in the range of 6.5 – 8.0. Protease activity was determined in relation to 2 substrates: casein and azokeratin.

In the studies on the influence of inhibitors on the activity of enzymatic preparations the following substances were used: phenylmethylsulfonyl fluoride (PMSF), N-ethylmaleimide (NEM) and ethyleno-bis-(etoxi-amino)

acid N,N,N,'N'-fouracetic (EGTA) all manufactured by "Sigma" in the concentration of 1 and 10 mM. Enzymatic material in the amount of 0.5 cm^3 was pre-incubated with 0.5 cm^3 of inhibitor solution for 30 min at 37°C. The same enzyme in buffer (28 mM phosphate buffer or Tris-HCl pH 7.8) was used as control. After incubation the reaction mixture was dialysed in the presence of buffer for 12 hours at the temperature of +4°C. In order to study reversibility of the reaction with EGTA, samples were pretreated with 0.5 cm^3 of 5 times higher concentration of Ca^{+2} (5 and 50 mM, respectively). Proteolytic activity was determined in the samples using casein and azokeratin as substrates. Final results were expressed in % activity assuming 100% as enzymatic activity of samples without inhibitor.

Results were presented as a mean of 3–5 independent repetitions for which 2–3 measurements were carried out. Experiments were repeated twice.

RESULTS

Keratinolytic activity of after-culture filtrates and homogenats

While looking for keratinolytic enzymes in the after-culture medium of the several keratinophilic species (K o r n i ł ł o w i c z-K o w a l s k a 1997a), it was found that their activity in relation to native feather keratin was low. It directed our research towards looking for the keratinolytic enzymes also in the mycelium. Results presented in Table 1 suggest that both homogenates obtained from whole hyphae (fraction H_1), and homogenates from the surface structures of fungi cells (fraction H_2) showed the ability for "digesting" feather

T a b l e 1

Comparison of enzymatic activities of after-culture filtrates (P) and mycelium homogenates (H) in relation to native feather keratin (in enzymatic activity U mg^{-1} of protein – mean values from two experiments)

Fungi	Days of incubation								
	7			14			21		
	P	H_1*	H_2**	P	H_1	H_2	P	H_1	H_2
A. quadrifidum str. 1	26.0	2.0	2.4	11.2	11.8	5.0	2.8	0	1.7
A. curreyi str. 2	33.0	11.9	4.8	7.1	15.0	11.3	16.6	8.3	21.7
Ch. pruinosum str. 1	28.0	1.1	0.72	19.3	8.8	2.75	13.0	7.4	2.4

Explanations: * Extracellular fraction, ** cell structure fraction

protein. However, their enzymatic activity was also low, even when compared to the activity of after-culture filtrates.

In the course of the present study it was noted that keratinolytic activity of after-culture filtrates and mycelium homogenates measured in relation to feathers as a natural substrate, can be the effect of hydrolysis of non-keratin proteins present in this substrate (M e r c e r 1958). For the same reason enzymatic activities of both fractions in relation to chemically pure keratin was determined at the same time (Table 2). Release of peptide substances confirmed keratinolytic properties of mycelial and extracellular proteases of the studied keratinophilic fungi. According to the opinion by N i c k e r s o n and D u r a n d (1963) keratinases is an enzyme responsible for the peptide release from keratin. Similarly as in the case of utilization of feathers as a substrate, a higher enzymatic activity in relation to pure keratine, was shown by the after-culture filtrates than mycelium homgenates (Table 2).

T a b l e 2

Enzymatic activity of after-culture filtrates (P) and mycelium homogenates (H_1) in relation to pure keratin as a substrate (in μg of released peptides of mg^{-1} of protein – mean values from two experiments)

Fungi	Days of incubation					
	7		14		21	
	P	H_1	P	H_1	P	H_1
A. quadrifidum str. 1	27	5	30	26	92	27
A. curreyi str. 2	19	6	38	15	104	14
Ch. pruinosum str.1	56	6	70	13	135	8

Some properties of crude preparations of fungi proteases deposing waste feathers

K e r a t i n a s e a c t i v i t y. The activity of this enzyme was determined in relation to pure keratin as a substrate in the crude enzymatic preparations (fraction after ultra filtration) (Table 3). An increase of enzymatic activity after separation of low-molecular compounds was expected. This effect was clearly visible in the case of mycelial keratinase. Whereas, partial purification of exokeratinase increased the activity of this enzyme to a lesser degree, and in some case its decrease was noted (Table 2 and 3).

S u b s t r a t e a f f i n i t y. The studies conducted in the framework of the present experiment discovered a wide spectrum of substrate saprophytic

Table 3

Activity of crude extracellular (Pm) and intracellular enzyme preparations (H_1m) in relation to pure keratin (in μg of released peptides of mg^{-1} of protein – mean values from two experiments)

Fungi	Days of incubation					
	7		14		21	
	Pm	H_1m	Pm	H_1m	Pm	H_1m
A. quadrifidum str. 1	74	72	165	221	51	224
A. curreyi str. 2	67	52	58	45	44	237
Ch. pruinosum str.1	89	66	171	209	59	139

Table 4

Activity of crude preparations of fungi proteases in relation to some globular and fibrous proteins (in enzymatic activity U mg^{-1} of protein – mean values from two experiments)

Substrate	Days of incubation	*A. quadrifidum* str. 1		*A. curreyi* str. 2		*Ch. pruinosum* str. 1	
		Pm	H_1m	Pm	H_1m	Pm	H_1m
Globular proteins							
Albumin	7	19	83	102	36	83	73
	14	25	25	84	41	242	73
	21	16	6	31	31	17	23
Hemoglobin	7	49	53	39	54	85	143
	14	32	33	48	18	89	62
	21	19	12	19	16	45	42
Casein	7	96	170	398	152	176	124
	14	166	158	248	81	293	186
	21	157	48	177	132	119	57
Fibrous proteins							
Azocoll	7	25	23	10	51	25	24
	14	95	68	82	121	107	106
	21	61	29	127	103	107	82,5
elastin	7	5,0	7,3	3,8	11,6	15	58
	14	16,6	23,4	24,5	17,4	47	20
	21	9,2	14.3	27,3	23,9	24	8
azo-keratin	7	3,8	0,3	4,1	2,4	2,4	3,0
	14	0,8	0,7	2,4	1,0	1,6	1,4
	21	1,4	0,6	3,8	3,8	4,1	3,0
chicken feathers	7	5	2,9	3,0	2,1	13	10,5
	14	1,5	1,0	2,0	4,6	3,5	3,5
	21	2,4	2,5	1,3	5,4	1,5	4,4
guinea pig hair	7	0,7	0,5	1,1	0	0,1	0
	14	0	0,6	0	0	0	0
	21	0	0,3	0	0	0	0

Explanation: extracellular Pm and mycelium fraction (H_1m)

proteases of keratinophilic fungi. It was found out that enzymatic preparations obtained (both fractions) hydrolysed globular protein easier than fibrous proteins (Table 4). Among the studied substrates casein and azocoll was "digested" the quickest, and native feathers the slowest, whereas guinea pig's hair was not decomposed, which agrees with the earlier observations by the present author (K o r n i ł ł o w i c z 1994).

In the present study it was observed that the enzymes synthetized in the initial period of feather lysis (1st-2nd week) usually showed higher activity in relation to fibrous proteins, i.e. azocoll and elastin. It should be noted, however, that in relation to azokeratin and native feathers enzymatic preparations obtained from the 7-days old cultures were most effective (Table 4).

The results obtained confirm a generally higher activity of extracellular proteases than mycelial ones. An exception to this was a strain of *A. quadrifidum*, that was characterized by a variable activity of these enzymes during culturing.

O p t i m u m pH. Study materials obtained from the 21-days old cultures of strain No.1 *Ch. pruinosum* were crude enzyme preparations released into the medium (Pm) and deposited in the mycelium (H_1m). Total proteolytic enzyme activity was determined in relation to casein, and their keratinolytic activity relation to azokeratin.

It was found that the studied enzymatic fractions showed more than one optimum of caseino- and keratinolytic activity (Fig.1). The fact that optimum of the extracellular and mycelial proteases were localized in the same pH range (7—8), which pointed to the neutral of alkaline character of these enzymes.

I n f l u e n c e o f p r o t e a s i s i n h i b i t o r s. In the present studies crude enzymatic preparations of *A. quadrifidum*, strain No. 1 and *Ch. pruinosum*, strain No. 1 obtained form the 21-days old cultures of these fungi were utilized.

Results (Table 5) point to a clear inhibition of proteases activity of the studied fungi by PMSF — serine proteinase inhibitor. The inhibitor used exert at the same time stronger influence on the the caseino- than keratinolytic activity. A less specific reagent was NEM — an inhibitor of tio-dependent enzymes. Whereas in the case of EGTA the activity of crude preparations of fungi proteases was inhibits only at higher concentrations (10 mM). Moreover, the above mentioned chelator exerted stronger influence on the activity of extracellular enzymes than on the mycelial enzymes. Similarly as in the case of PMSF the effect just described concerned the caseinolytic activity to a higher degree. From the data in Table 5 it follows that Ca ions had little contribution to make the reaction with EGTA reversible.

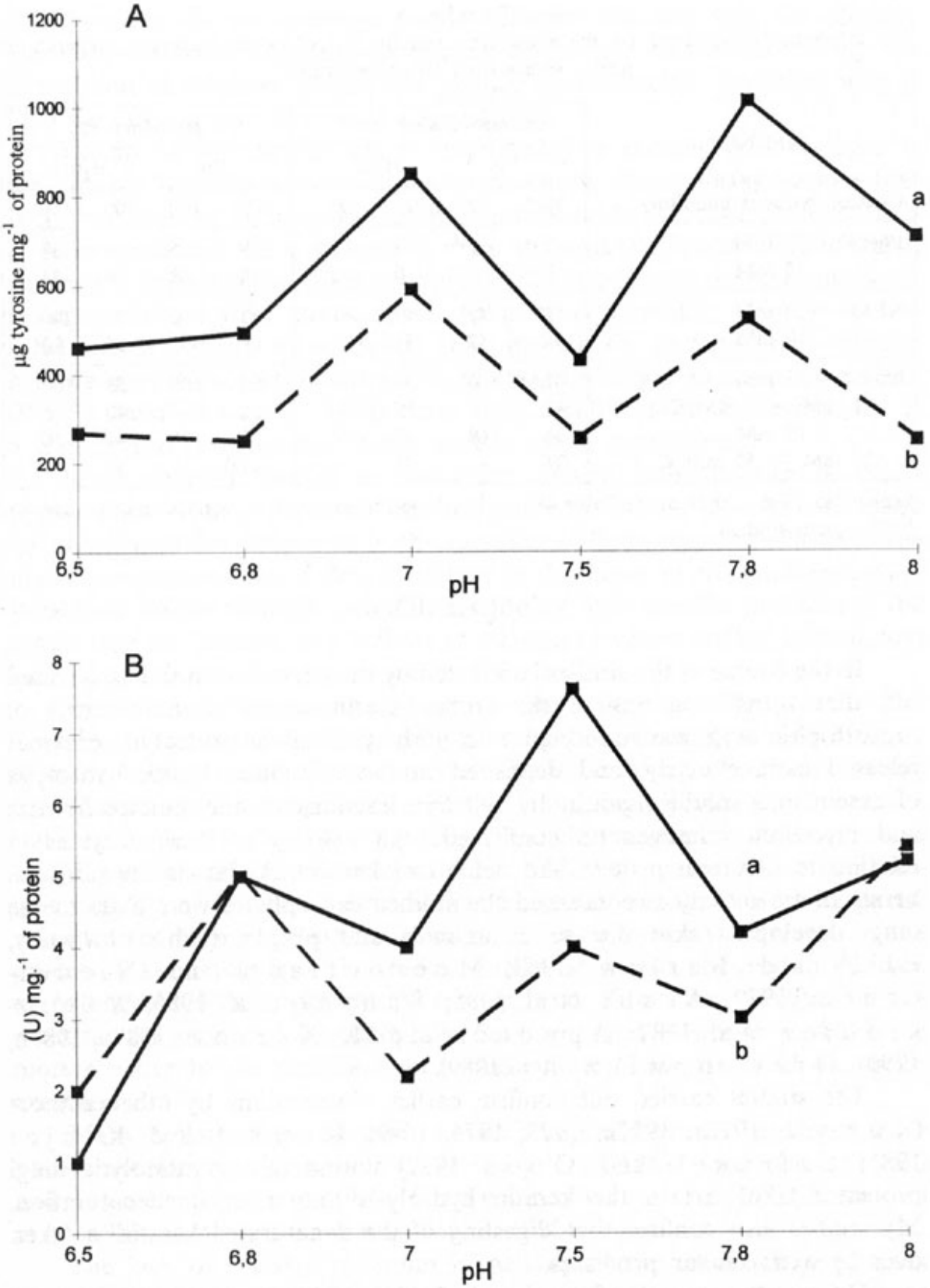

Fig. 1. Influence of pH on the activity of raw proteasis preparations of *Chrysosporium pruinosum* strain 1 (mean values from two experiments) in relation to casein (A) and azokeratin (B): a – extracellular; b – mycelium fraction

Table 5

The influence of inhibitors on the proteolytic activity (%) of crude enzymatic preparations (mean values from two experiments)

Inhibitor	*A. quadrifidum* str. 1				*Ch. pruinosum* str. 1			
	Pm		H_1m		Pm		H_1m	
Control (without inhibitor)	100[a]	100[b]	100	100	100	100	100	100
PMSF – 1 mM	38[a]	49[b]	14	15.5	9	65	4	37
10 mM	0	17.5	0	2	0	38	0	34
NEM – 1 mM	57[a]	45[b]	91.5	40	81	100	72	100
10 mM	36	42	81	37	64	87	56	92
EGTA – 1 mM	47.5[a]	100[b]	71.5	67	93	100	58	100
1 mM + 5 mM Ca^{2+}	42	–	97	–	118	–	40	–
10 mM	34	69	37	57	11.5	35	44	10
10 mM + 50 mM Ca^{2+}	34	–	37	–	11.0	–	31	–

Explanation: Pm and H_1m see Table 4: a – in relation to casein, b – in relation to azokeratin, "–" – non studied

DISCUSSION

In the course of the studies conducted by the present author, it was found out that transformations of the crude keratin waste in the cultures of saprotrophic fungi are connected with both synthesis of proteolytic enzymes released extracellularly, and deposited in the mycelium. Quick hydrolysis of casein and soluble keratin by cell free fractions of after-culture filtrates and mycelium homogenates confirmed high activity of these enzymes in relation to common protein and denatured keratin. As far as the range of keratinolytic activity is concerned the studied saprophytes were close to the fungi developing skin disease in animals and people (Chattaway, Ellis and Barlow 1963; Meevootison and Niederpruem 1979; Asahi et al. 1985; Sanyal et al. 1985; Wawrzkiewicz et al. 1987; Apodaca and Mc Kerrow 1989a, 1989b, 1990; Grzywnowicz et al.1989).

The studies carried out confirm earlier observations by other authors (Kunert 1972a, 1972b, 1973, 1976, 1989; Kunert and Krajci 1981; Safranek and Goos 1982) stating that keratinolytic fungi proteases take part in the keratin hydrolysis only after its denaturation. My studies also confirm that digesting of the denaturated keratin is taken over by extracellular proteases.

During the period of maximum feather lysis (3 weeks old cultures) activity of these enzymes (after-culture filtrates) in relation to pure keratin was clearly increasing. Whereas, the activity of mycelial enzymes (mycelium

homogenates) did not undergo major changes. The fact that the changes recorded concerned keratine component of feathers was confirmed by the stimulation of sulphate release and peptide accumulation correlated with it (Korniłłowicz-Kowalska 1997a).

Results of our studies allow for drawing a conclusion that there is a enzymatic "cascade" at work in the fungi cultures decomposing feathers that is characterized by an increasing affinity in relation to substrate proteins. This opinion agrees with Hose and Evans' thesis (1977) according to which enzymes of dermatophytes that take part in the hair keratin decomposition are more specialized than the enzymes splitting non-keratin proteins of this substrate. It cannot be excluded that proteolytic enzymes that catalise hydrolysis of feather protein are also of common origin as some researchers (Page and Stock 1974) state that keratinase of dermatophytes is a transformed protease that takes part in spore germinating.

More detailed studies on substrate affinity with the use of crude preparations of fungi proteases that are produced during the phase of quick lysis confirmed the differences in the activities of these enzymes related to the age of the culture, with a clear similarity in the range of substrate spectrum. It became visible through quick hydrolysis of little specific proteins of the casein type and azocoll, low activity in relation to native feather keratin, and lack of abilities to decompose native hair keratin.

Even though the results of author's own studies clearly point to the disability of the protease of saprotrophic fungi for independent solubilization of crude keratin waste which give basis for undertaking an attempt to use preparations of these enzymes as additives improving digestibility of commercial meal made of feathers that are characterized by a very low index of amino acid digestibility (Normy Żywienia Drobiu, 1972). Moreover, higher keratinolytic activity of unrefined extracellular proteases in comparison to partly refined preparations suggest that low-molecular compounds take part in the process of keratinolysis of the studied fungi that is also observed in the case of pathogenic dermatophytes (Weary and Canby 1969). For the practical utilization of keratinophilic fungi enzymes it would probably be more effective to use non-fractioned after-culture filtrates instead of refined keratinase.

Many authors drew attention to the complex nature of proteases from pathogenic dermatophytes (Chattaway et al. 1963; Meevootison and Niederpruem 1979; Grzywnowicz et al. 1989; Sanyal et al. 1989; Łobarzewski et al. 1990).

The lack of decisive optimum of activity of the crude *Chrysosporium pruinosum* proteases preparations noted by the present authors, pointed to a similar phenomenon in relation to keratinolytic saprophytic fungi. The pH range in which maxima of enzymatic activities were placed and

inhibition by PMSF confirmed that the proteases of the studied fungi belonged to neutral and alkaline groups of serine proteases. Proteases of pathogenic dermatophytes belong to these groups as well (Chattaway et al. 1963; Meevootison and Niederpruem 1979; Takiuchi et al. 1984; Asahi et al. 1985; Sanyal et al. 1985; Apodaca and Mc Kerrow 1989; Łobarzewski et al. 1990; Qin et al. 1992). It can also be assumed that proteolytic enzymes of at least some of the keratinolytic saprophytes are dependent on metals in analogically to some skin pathogens (Łobarzewski et al. 1990). It could also be confirmed by a considerable sensitivity of *Ch. pruinosum* enzymes to EGTA – a factor that is chelating Ca ions and the lack of reversibility of reaction under the influence of this cation. Generally lower sensitivity to the *Arthroderma quadrifidum* enzyme inhibitors used than *Ch. pruinosum* enzymes suggested higher differentiation of the proteases form geophilic dermatophytes' complex than form *Chrysosporium*. This fact can be significant in the survival of these fungi on the keratin "chips" in the soil. It is well known that microorganism with a more differentiated enzymatic apparatus are characterized by a higher abilities for competition and adaptation in the environment that the microorganisms handicapped in this respect.

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Badania nad rozkładem odpadów keratynowych przez saprotroficzne mikromycetes. III. Aktywność i właściwości enzymów keratynolitycznych

Streszczenie

W prezentowanej pracy wykazano, że przemiany keratyny odpadowej (pióra kurcząt) w hodowlach grzybów keratynofilnych są związane z biosyntezą zewnątrzkomórkowych oraz zlokalizowanych w grzybni enzymów proteolitycznych. Surowe preparaty tych enzymów łatwo hydrolizowały białka globularne (zwłaszcza kazeinę), azokoll oraz zdenaturowaną keratynę, wykazując niższą aktywność w stosunku do natywnej keratyny. Stwierdzono, że proteazy saprofitycznych grzybów keratynolitycznych reprezentowały grupę proteaz serynowych o optimum aktywności w zakresie odczynu obojętnego lub lekko alkalicznego oraz charakteryzowały się szerokim spektrum substratowym.