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Research article

Degradation of keratin substrates by keratinolytic fungi

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ABSTRACT

Background: The hydrolysis of keratin wastes by microorganisms is considered a biotechnological alternative for recycling and valorization through keratinolytic microorganisms. Despite their resistant structure, keratin wastes can be efficiently degraded by various microorganisms through the secretion of keratinases, which are promising enzymes for several applications, including detergents, fertilizers, and leather and textile industry. In an attempt to isolate keratinolytic microorganisms that can reach commercial exploitation as keratinase producers, the current work assesses the dynamics of keratin biodegradation by several keratinolytic fungal strains isolated from soil. The activity of fungal strains to degrade keratin substrates was evaluated by SEM, FTRIR-ATR spectra and TGA analysis.
Results: SEM observations offered relevant information on interactions between microorganism and structural elements of hair strands. FTIR spectra of the bands at 1035–1075 cm^{-1} assigned to sulfoxide bond appeared because of S–S bond breaking, which demonstrated the initiation of keratin biodegradation. According to TGA, in the second zone of thermal denaturation, where keratin degradation occurs, the highest weight loss of 71.10% was obtained for sample incubated with *Fusarium* sp. 1A.
Conclusions: Among the tested strains, *Fusarium* sp. 1A was the most active organism in the degradation process with the strongest denaturation of polypeptide chains. Because keratinolytic microorganisms and their enzymes keratinases represent a subject of scientific and economic interest because of their capability to hydrolyze keratin, *Fusarium* sp. 1A was selected for further studies.

1. Introduction

Keratin is an insoluble fibrous protein characterized by high stability due to the high degree of cross-linkages by disulfide and hydrogen bonds. It also contains a variety of amino acids, predominantly cystine, lysine, proline, and serine. Considering the secondary structural conformation, keratins have been classified into α- (α-helix of hair and wool) and β-keratins (β-sheets of feather) [1,2]. In addition, keratins are grouped into hard keratin (hair, feather, nails, wool, etc.) having a high disulfide bond content and soft keratin (skin) with a low disulfide bond content. Keratin-rich wastes are troublesome environmental contaminants and are released in increasing quantities as byproducts from agro-industrial processes in the form of feathers, hair, nails, and horns. Fungi play an ecological role in the degradation of keratin substrates through their contribution to recycling the carbon, nitrogen, and sulfur from keratins. Considering from both economic and environmental point of view, attention was focused on the management of recalcitrant keratinous wastes. The hydrolysis of keratin wastes by microorganisms is considered a biotechnological alternative for recycling and valorization through keratinolytic microorganisms. Keratinases, considered as proteases with keratinolytic function, act synergistically with other keratinolytic enzymes to degrade the complex supramolecular organization of keratin [3,4]. After disrupting the disulfide bonds of cysteine, the major amino acid in keratin, the keratin substrate is more easily available to the hydrolytic enzymes secreted by microorganisms.

Despite the resistant structure, keratin wastes can be efficiently degraded by various microorganisms that secrete of keratinolytic enzymes, such as keratinases, which are a group of serine or metalloproteases. These enzymes are predominantly extracellular and are produced by microorganisms growing in a basal medium containing keratinous substrates [5]. It is believed that in the future, microbial keratinases will occupy a special niche among proteases as valuable enzymes for the bioprocessing of the keratinous wastes, which are released into the environment in huge amounts because of human activities [6,7,8,9]. Because of their better performance

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due to higher specificity and the keratinous waste biomass available, keratinases will replace proteases in the leather industry and detergents [10].

Many microorganisms grow naturally on keratinous materials. Among bacteria, keratin biodegradation has been demonstrated by *Bacillus*, particularly *Bacillus licheniformis* [11,12,13] and *Bacillus subtilis* [14,15,16], and *Chryseobacterium* [17,18,19]. Moreover, actinomycetes from the *Streptomyces* genus are known to produce keratinases [20,21]. The most common active keratinolytic fungi belong to *Aspergillus* [22,23], *Penicillium* [24,25], *Fusarium* [26] *Microsporum* [27], *Trichoderma* [28], and *Chrysosporium* genera [29,30]. A relevant review on the microbiological deterioration of keratinous substrates was done by Blyskal [31], who listed and cataloged representative and predominant fungal genera and highlighted the most active ones.

In an attempt to isolate keratinolytic microorganisms that can reach commercial exploitation as keratinase producers, the current work asses the dynamics of keratin biodegradation by several keratinophilic fungal strains isolated from soil. The ability of fungal strains to degrade horse hair keratin was evaluated by scanning electron microscopy (SEM), Fourier transform infrared (FITR) spectroscopy, and thermogravimetric analysis (TGA).

2. Material and methods

2.1. Chemicals and reagents

Chemicals and reagents were purchased as follows: $ZnSO_4 \cdot 7H_2O$ from Merck, Germany; K_2HPO_4 from UCB, UK; $CaCl_2$ and $FeSO_4$ $7H_2O$ from Reactivul, Romania.

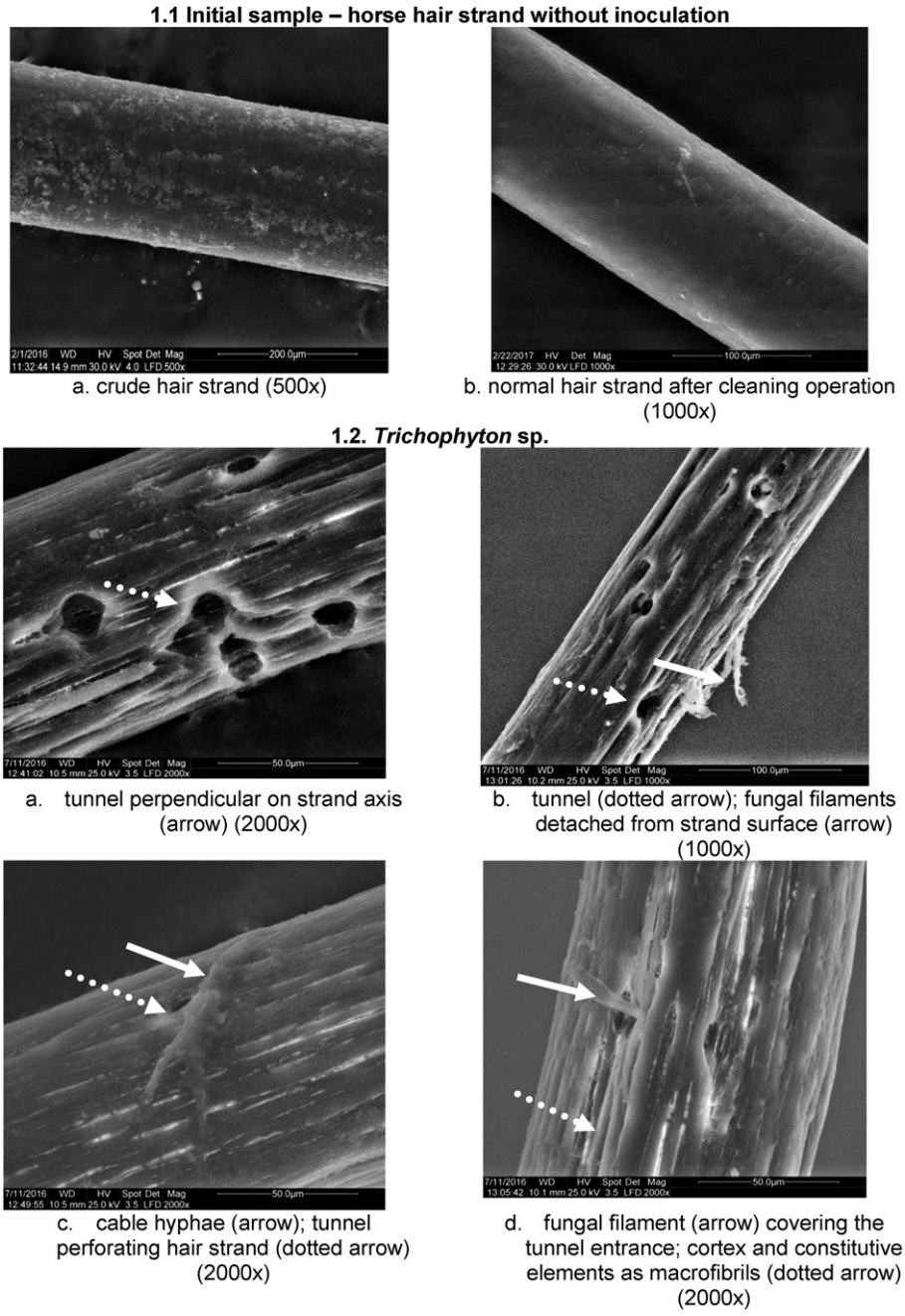


Fig. 1. SEM images of horse hair strands incubated with keratinolytic fungi grown on agitated liquid cultures (micrographs of 21-d culture). 1.1. Initial sample without inoculation; 1.2. *Trichophyton* sp.; 1.3. *Fusarium* sp. strain 1A; 1.4. *Trichoderma* sp.; 1.5.*Cladosporium* sp.; 1.6. *Microsporum* sp.; 1.7. *Fusarium* sp.; 1.8. *Phytophthora* sp.; 1.9. *Chrysosporium* sp.

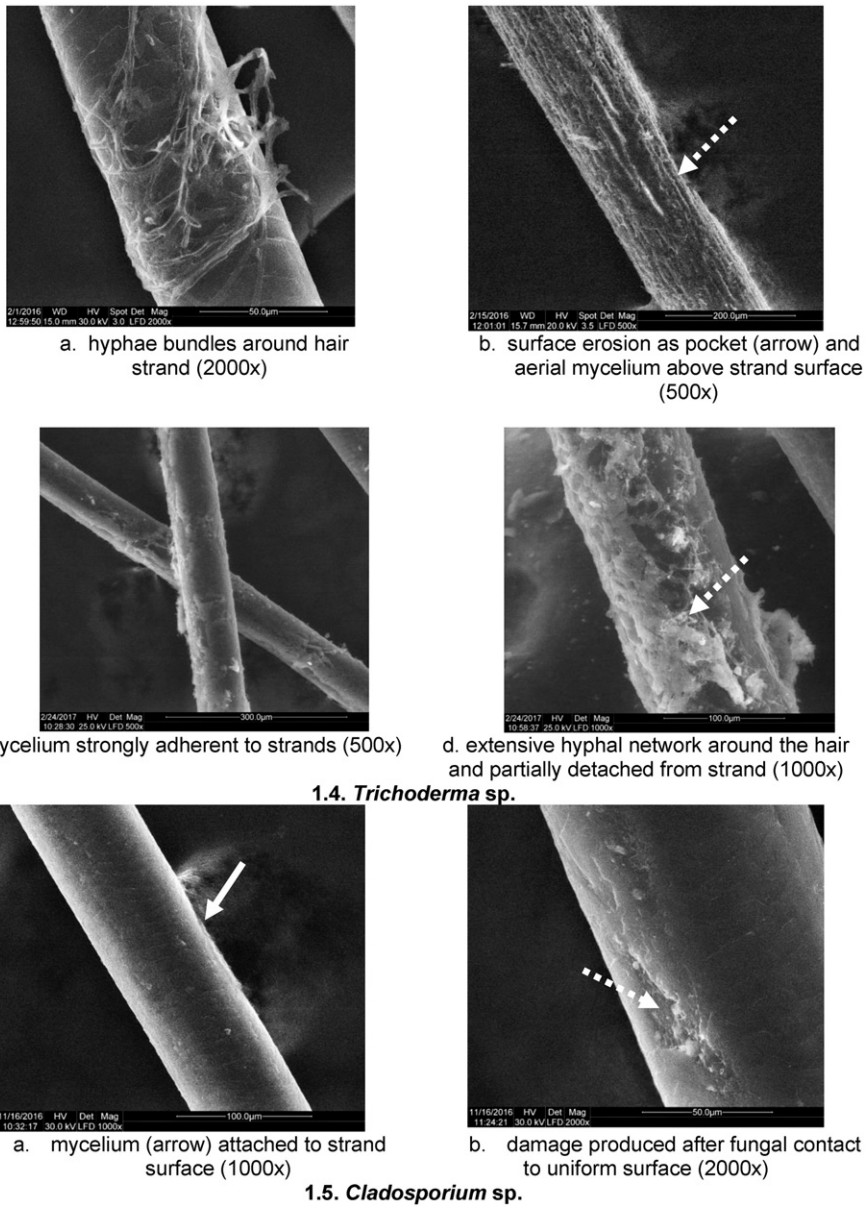


Fig. 1 (*continued*).

2.2. Microorganisms

The experiments were conducted using the following keratinophilic fungal strains isolated from soil: *Trichophyton* sp., *Fusarium* sp. strain 1A, *Trichoderma* sp., *Cladosporium* sp., *Microsporum* sp., *Fusarium* sp., *Phytophthora* sp., and *Chrysosporium* sp. The strains belonged to Microbial Collection of INCDCP-ICECHIM and were maintained on potato dextrose agar (PDA) slants at 4°C.

2.3. Keratin substrate

Horse hair strands obtained from a local household were surface sterilized with ethanol (3%), washed thoroughly under running water, and then dried at 60°C. The hair strands were cut into pieces of about 2 cm in length, separated into portions of 50 mg weight, and autoclaved at 121°C for 20 min (Panasonic MLS-3751L).

2.4. Conditions of cultivation

To study the time course for microbial growth and substrate degradation, the selected microorganisms were cultured in 250-ml Erlenmeyer flasks containing 50 ml medium and 50 mg keratin substrates. The flasks were inoculated with a piece of mycelium from each strain. A basal solution with the following composition was used (g/l): 0.1, KH_2PO_4; 0.01, $CaCl_2$; 0.1, $FeSO_4$ $7H_2O$; 0.005, $ZnSO_4$ $7H_2O$; pH = 7.5. The control contained only the basal mineral solution and keratin substrate, without any microorganism. All the flasks were incubated on a rotary shaker (Heidolph Unimax 1010) at 100 rpm and 27 ± 1°C for 21 d. The cultivation experiments were performed in triplicate. The fungal cultures were filtered through Whatman filter paper No. 1. The strand fragments were washed gently to remove fungal hyphae, dried at 60°C for 48 h, and analyzed by FTIR and TGA. The strand fragments with mycelium were analyzed by SEM.

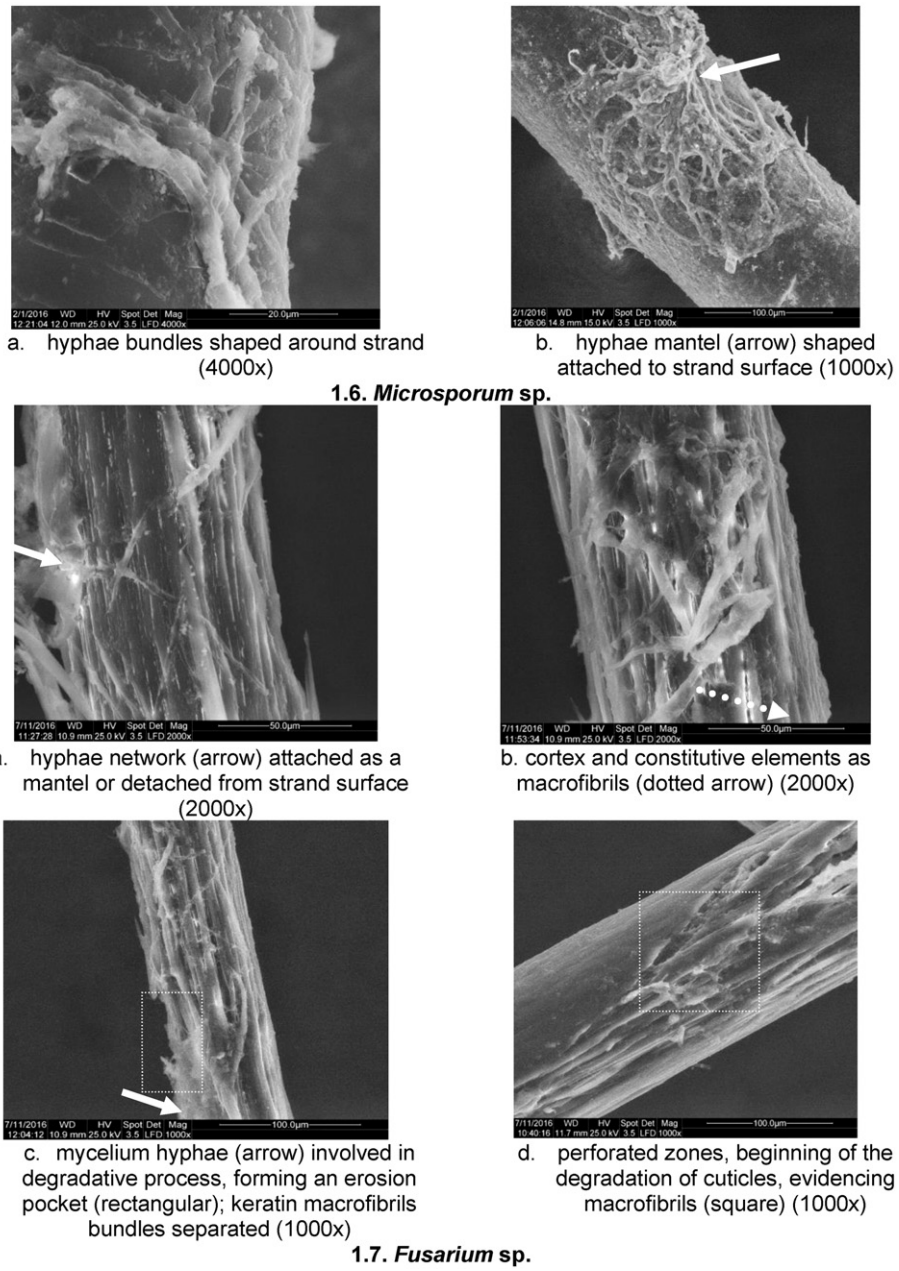


Fig. 1 (*continued*).

2.5. Morphological analysis (SEM)

Morphological analysis of samples was carried out by SEM performed on a FEI-QUANTA 200 instrument in ICECHIM. The horse hair strand samples recovered from the culture medium of the biodegradation experiments were placed on metallic support, an aluminum standard stub, using a double-sided adhesive carbon tape. SEM images were obtained at an accelerating voltage of 20–30 kV and 133 Pa using a large field detector (LFD). Micrographs of the samples were investigated at different magnifications to identify changes after the degradation process. For each sample, 8–10 micrographs were performed and the relevant images were obtained.

2.6. FTIR spectra

FTIR was performed to evaluate the biodegradation extent. FTIR spectra were acquired using a FTIR Tensor 37 spectrometer (Bruker) with ATR Golden Gate in the ICECHIM laboratory. All spectra were recorded from 500 to 4000 cm^{-1}.

2.7. Thermal stability

The thermal stability of the hair samples recovered from culture medium of biodegradation experiments was investigated by TGA using a TGA Q5000IR (TA instruments) in ICECHIM. Samples (50–10 mg) were heated from 20°C to 700°C at a 10°C/min scanning rate under nitrogen atmosphere (flow rate, approximately 200 mL min). The onset temperature was determined as the temperature corresponding to the crossover of tangents drawn on both sides of the decomposition trace, and the residue was evaluated as the residual weight at 700°C.

3. Results and discussions

The experiments were conducted using keratinolytic fungal isolates from soil samples because of the accumulation of keratin wastes in the

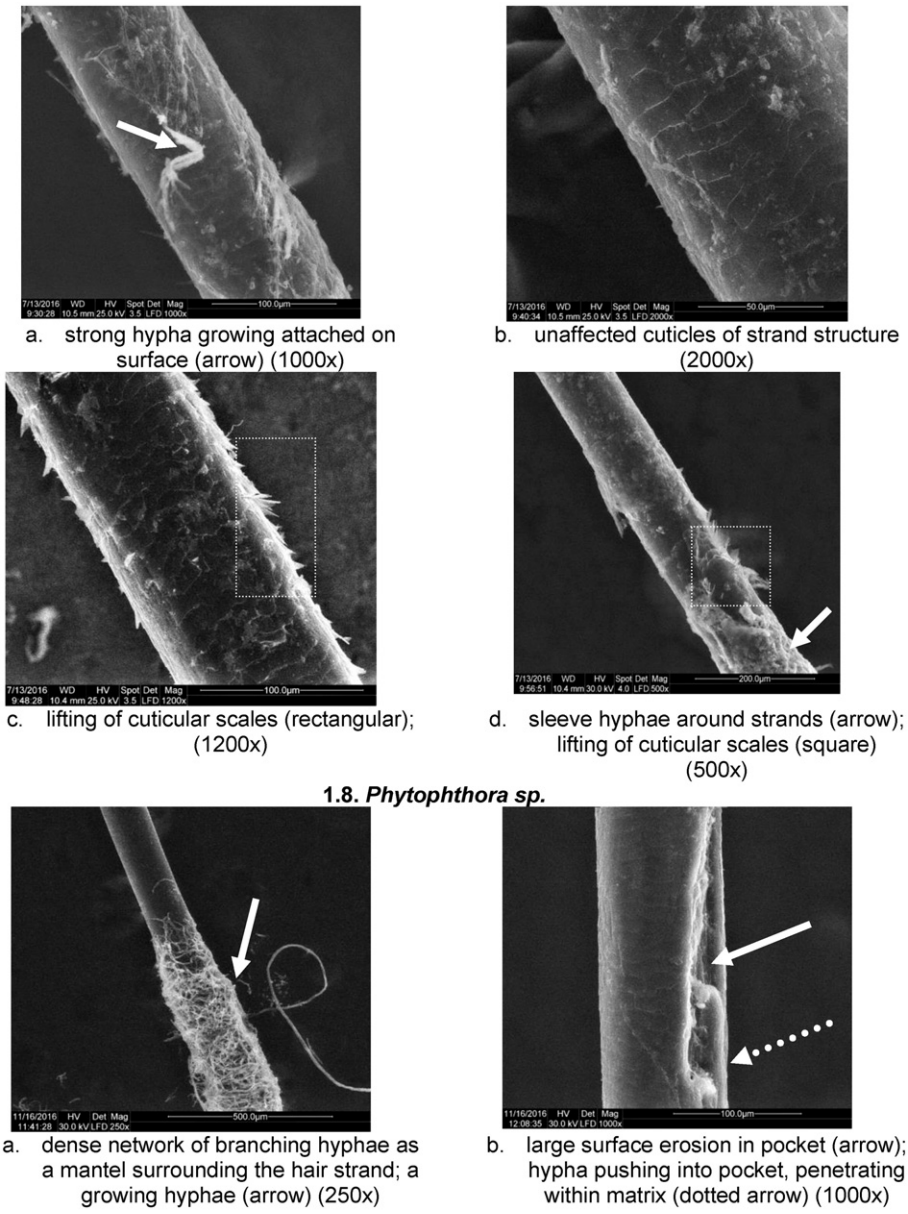


Fig. 1 (*continued*).

soils (geophilic). The fungal isolates were identified up to genus level on the basis of morphological characteristics (data not shown). Identification of species is currently in progress.

3.1. Morphological analysis (SEM)

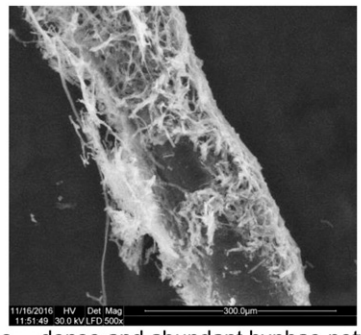
The morphology of the strand samples was investigated by SEM, which offered detailed information on the interactions between microorganism and structural elements of the hair strand. The hair strand consists of three main morphological components: cuticle, cortex, and medulla. These layers are held together by the cell membrane complex. The cortex, which is responsible for the great tensile strength of the hair fiber, is made of microfibrils. Filaments are packed together and contain organized α-helical rods of keratin, which are embedded in an amorphous matrix. The cortex is covered with an external cuticle, which acts as a protective barrier against destruction. The medulla consists of specialized cells that contain air spaces [32].

The biodegradation of keratin by keratinolytic fungi occurs in a model with two basic forms of attack: surface erosion followed by radial penetration. The keratinolytic activity varied within species of the same genus and, in some cases, even within strains of the same specie [33,34].

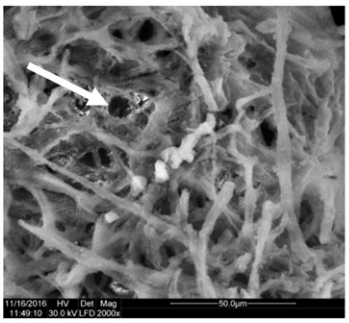
Hair samples after 21 d of incubation with fungal strains were analyzed by SEM, and the most representative micrographs of hair horse strand are presented in Fig. 1. Below each image, a detailed explanation on the elements of fungal growth and/or effects produced by fungi on horse hair strand structure is given.

Fig. 1.1 shows the initial samples of horse hair strands before (1.1a) and after the cleaning operation (ethanol sterilization, washing with water, and drying) (1.1b). Fig. 1.1 also shows the mycelium that strongly adhered to the hair strands (1.1c) and the extensive hyphal network around the hair that was partially detached from the strand (1.1d).

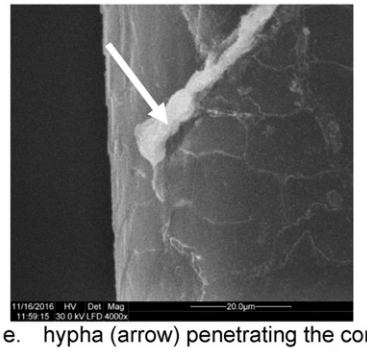
SEM images of *Trichophyton* sp. (Fig. 1.2) revealed many features: perforating tunnels perpendicular to strand axis (Fig. 1.2a), few fungal filaments detached from strand surface (Fig. 1.2b), strong cable hyphae

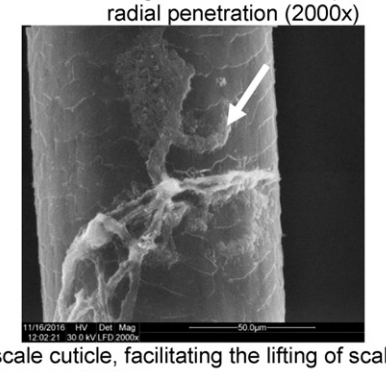


c. dense and abundant hyphae network covering hair strand (500x)



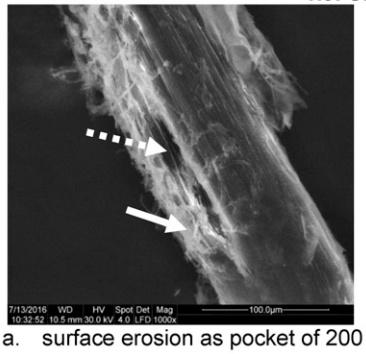
d. dense network of branching hyphae on strand surface; cavity in branching indicating the entrance in a point of radial penetration (2000x)



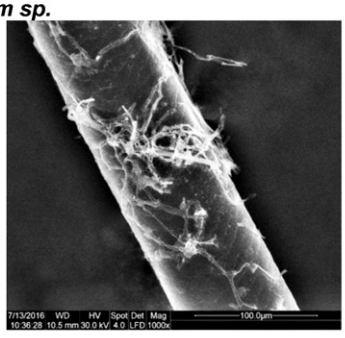


e. hypha (arrow) penetrating the cortex below scale cuticle, facilitating the lifting of scales cuticle (left, 4000x) (right, 2000x)

1.9. *Chrysosporium sp.*



a. surface erosion as pocket of 200 um length (arrow) surrounded by eroding mycelium; hypha crossing the pocket (dotted arrow) (1000x)



b. hyphae network around hair strand; detached hyphae (1000x)

Fig. 1 (*continued*).

(Fig. 1.2c) growing near a penetrating tunnel (Fig. 1.2d), a fungal filament covering the tunnel entrance, and the deterioration of hair strands exposing the cortex and constitutive elements such as macrofibrils (Fig. 1.2e). Because *Trichophyton* belongs to dermatophytes, the fungal strain produced the perforation of hair strands, showing structures such as tunnels, which are strong destructors of native keratins, therefore rapidly degrading the substrate.

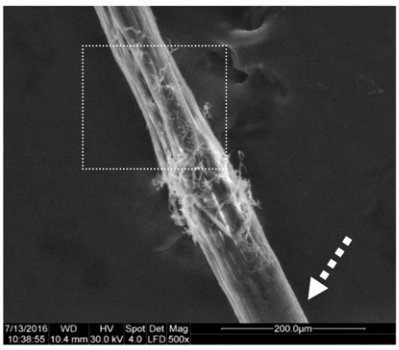
Fusarium sp. strain 1A (Fig. 1.3) showed hyphae bundles growing around hair strands (Fig. 1.3a). This strain could destroy the integrity of hair strand, producing surface erosion as a "pocket" (Fig. 1.3b), a term explained by Marchisio et al. [33].

Trichoderma sp. (Fig. 1.4) produced mycelium either attached to hair strand surface or in certain areas as partially aerial mycelium (1.4a). In addition, there was observable damage produced to uniform surface after contact with the fungal strain (Fig. 1.4b).

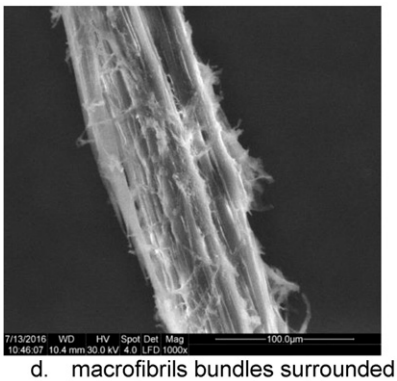
SEM images showed that *Cladosporium* sp. produced dense mantel-like hyphae around the hair strand (Fig. 1.5a) and produced a hyphae network similar to a mantel strongly attached to the hair strand (Fig. 1.5b).

Fig. 1.6 presents representative micrographs showing the interactions between *Microsporum* sp. and keratin substrate: hyphae network attached as a mantel to hair strand or detached from strand surface (Fig. 1.6a), deterioration of hair strand by exposing the cortex and constitutive elements such as macrofibrils (Fig. 1.6b), degradative effect of fungal mycelium by forming an erosion pocket and the separation of macrofibrils bundles (Fig. 1.6.c), and the beginning of cuticle degradation and evidence of macrofibril formation (Fig. 1.6d).

In SEM images of the sample incubated with *Fusarium* sp., lifting of cuticular scales can be observed, which is a prerequisite step in the degradation of hair strand (Fig. 1.7a). An unaffected part of the hair strand with intact cuticles (Fig. 1.7b) was observed. Near the deteriorated zone, lifting of cuticular scales was observed (Fig. 1.7c), which was produced by the hyphae growing around the hair strand (Fig. 1.7d).



c. digested cuticle scales and macrofibriles (square); hyphae around strand producing the lifting of cuticle; intact structure of hair strand (arrow) (500x)



d. macrofibrils bundles surrounded by lax hyphae network; cuticles layer removed (1000x)

Fig. 1 (*continued*).

Microscopic observations of the sample incubated with *Phytophthora* sp. showed the following: dense network of branching hyphae growing as a mantel surrounding the hair strand and growing hyphae detached from the strand (Fig. 1.8a); large surface erosion in pocket and a hypha pushing into the pocket, penetrating the strand matrix (Fig. 1.8b); a strong hypha penetrating below cortex, facilitating the lifting of cuticlar scales (Fig. 1.8c and Fig. 1.8d).

In the sample incubated with *Chrysosporium* sp., surface erosion was observed as a pocket of 200 μm length, surrounded by eroding mycelia and a hypha crossing the pocket (Fig. 1.9a). Some filaments forming a network grew while attached around the hair strand, while others were detached from strand (Fig. 1.9b). In Fig. 1.9c along the hairline, it can be seen the different stages of hair degradation, from the intact structure of the hair strand to the digestion of cuticle scales and finally separation of macrofibrils. Fig. 1.9d shows macrofibril bundles surrounded by lax hypha network, exposed by the removing of strand cuticle after incubation with the fungal strain.

SEM images have shown that all the tested strains could develop adequate structures related to surface erosion and radial penetration. In our opinion, some SEM images reveal, for the first time, relevant aspects of hair strand degradation by certain strains, namely *Fusarium*, *Phytophthora*, and *Cladosporium*.

3.2. FTIR results

Infrared absorption spectra of horse hair strand after 21 d of incubation with fungal strains showed two large characteristic absorption bands, one assigned to the peptide bonds (–CONH–) and the other assigned to alkyl chain and OH bond (Fig. 2).

The bands positioned in the domain of 3300–3000 cm^{-1} are assigned to the H-bonded OH stretch of water present in keratin, while those at 3000–2700 cm^{-1} are assigned to the stretching modes of the lipid alkyl chains (methyl and ethyl).

The fundamental structural units of the polypeptide chain of keratin are known as amide I, II, and III. The characteristic amide A band occurred at 3270–3280 cm^{-1}. Amide I is related to C–O stretching and occurs at 1700–1600 cm^{-1}. Amide II is related to N-H bending and C–H stretching vibration and falls in 1540–1520 cm^{-1}. Amide III is related to a combination of C–N stretching and C–O bending vibration and occurs in the range 1220–1300 cm^{-1}. For the initial horse hair sample, the amide I, II, and III bands were visible at 1632 cm^{-1}, 1531 cm^{-1}, and 1234 cm^{-1}, respectively, in accordance with those reported in other papers [35,36,37].

The peptide bonds from keratin remained unaffected during the fungal degradation, and their position did not change. Only a small migration to smaller values for amide I and II and to higher values for amide III was observed. For example, the values of amide I decreased from 1632.76 cm^{-1} (control) to around 1631 cm^{-1} (sample incubated with *Trichophyton* sp., *Cladosporium* sp., and *Chrysosporium* sp.), 1630 cm^{-1} (*Microsporum* sp.), and 1629 cm^{-1} (*Trichoderma* sp. and *Phytophthora* sp.). The same behavior was recorded for amide II: from 1531.17 cm^{-1} (control) to around 1530 cm^{-1} (*Microsporum* sp.), 1528 cm^{-1} (*Chrysosporium* sp.), 1522 (*Fusarium* sp. strain 1A), 1520 cm^{-1} (*Trichophyton* sp.), 1519 cm^{-1} (*Trichoderma* sp. and *Phytophthora* sp.), and 1518 cm^{-1} (*Cladosporium* sp. and *Microsporum* sp.). In the case of amide III, the peaks migrated to higher values: from 1234,95 cm^{-1} (control) to around 1236 cm^{-1} (*Trichophyton* sp., *Trichoderma* sp., *Microsporum* sp., *Fusarium* sp., *Phytophthora* sp., and *Chrysosporium* sp.) and 1237 cm^{-1} (*Fusarium* sp. 1A).

During the biodegradation process, only the S–S bonds were affected by microorganisms, and after long contact, the microbial enzymes secreted disrupt the peptide bonds of the keratin chains. The bands assigned to sulfide bonds S–S occur in the range 600–620 cm^{-1} (not very clear in the presented spectra). Signs of biodegradation initiation are the appearance of the bands at 1035–1075 cm^{-1} assigned to sulfoxide bond (S═O) due to S–S bond breaking. The presence of the oxide forms of sulfur is important for the oxidation that occurs as monoxide-to-dioxide, then proceeding to full oxidation with the formation of cysteic acid.

The bands assigned to sulfoxide bonds are evident for all samples, with the highest peak areas obtained for sample incubated with *Fusarium* sp. strain 1A (Fig. 2c), *Microsporum* sp. (Fig. 2f), and *Chrysosporium* sp. (Fig. 2i). The band intensity decreased for samples incubated with *Cladosporium* sp. (Fig. 2e) and *Trichoderma* sp. (Fig. 2d). The peak areas of samples incubated with *Trichophyton* sp.) (Fig. 2b), *Fusarium* sp. (Fig. 2g) and *Phytophthora* sp. (Fig. 2h) were approximately equal and much lower than the peak areas of other samples.

3.3. TGA results

TGA was performed to quantify the thermal degradation of keratin in a controlled atmosphere. Exposure to increasing temperature led to the breaking of disulfide bonds, which ensured a protective layer for fibrils. The ordered structure of keratin is affected and changed to a disorganized one. It is considered that because covalent S–S bonds have the highest energy of all the bonds in the interface, the cleavage represents the rate-determining step of keratin degradation [38].

Table 1 and the graphic representations summarized in Fig. 3 indicate the presence of three main zones of weight losses, similar to data reported in others papers [38,39].

The first zone (0–150°C) is due to water evaporation. The second one (150–500°C) corresponds to the denaturation of polypeptide chain in keratin, which resulted in major weight loss. In the temperature domain of 280–350°C, keratin suffers organic degradation of microfibrils and matrix. The third zone (500–700°C) represents the complete degradation of keratin and hair structure. The carbonized residue was obtained after heating at 700°C.

Results of TGA profiles showed some differences in the characteristic behavior of keratin substrates after incubation with different fungal strains. Even water evaporation occurred at different temperatures and migrated from 87.7°C in control, to smaller values in samples 2 (78.6°C), 3 (78.6°C), 6 (45.3°C), and 9 (42.6°C), or to higher values in samples 4 (92.7°C), 5 (90.7°C), 7 (83.7°C), and 8 (81.6°C). For all samples, excluding the sample incubated with *Fusarium* sp. strain 1A, the amount of water loss increased when compared with the control sample (7.38%), and the maximum was reached in the sample incubated with *Chrysosporium* sp. (8.02%).

In the second zone of thermal denaturation, the highest weight loss (71.10%) was obtained for the sample incubated with *Fusarium* sp. strain 1A, while the sample incubated with *Chrysosporium sp.* reached the lowest value.

In the temperature domain of 150–500°C, we identified two stages of thermal degradation in the control and samples incubated with *Trichophyton*, *Trichoderma,* and *Cladosporium*, while samples incubated with *Fusarium* sp. strain 1A, *Microsporum*, *Fusarium*, *Phytophthor*a, and *Chrysosporium* exhibited three degradation stages. The peaks for samples with two stages were recorded at 237–242°C, with maxima around 297–303°C; these temperature values decreased for hair fibers after microbial incubation as compared

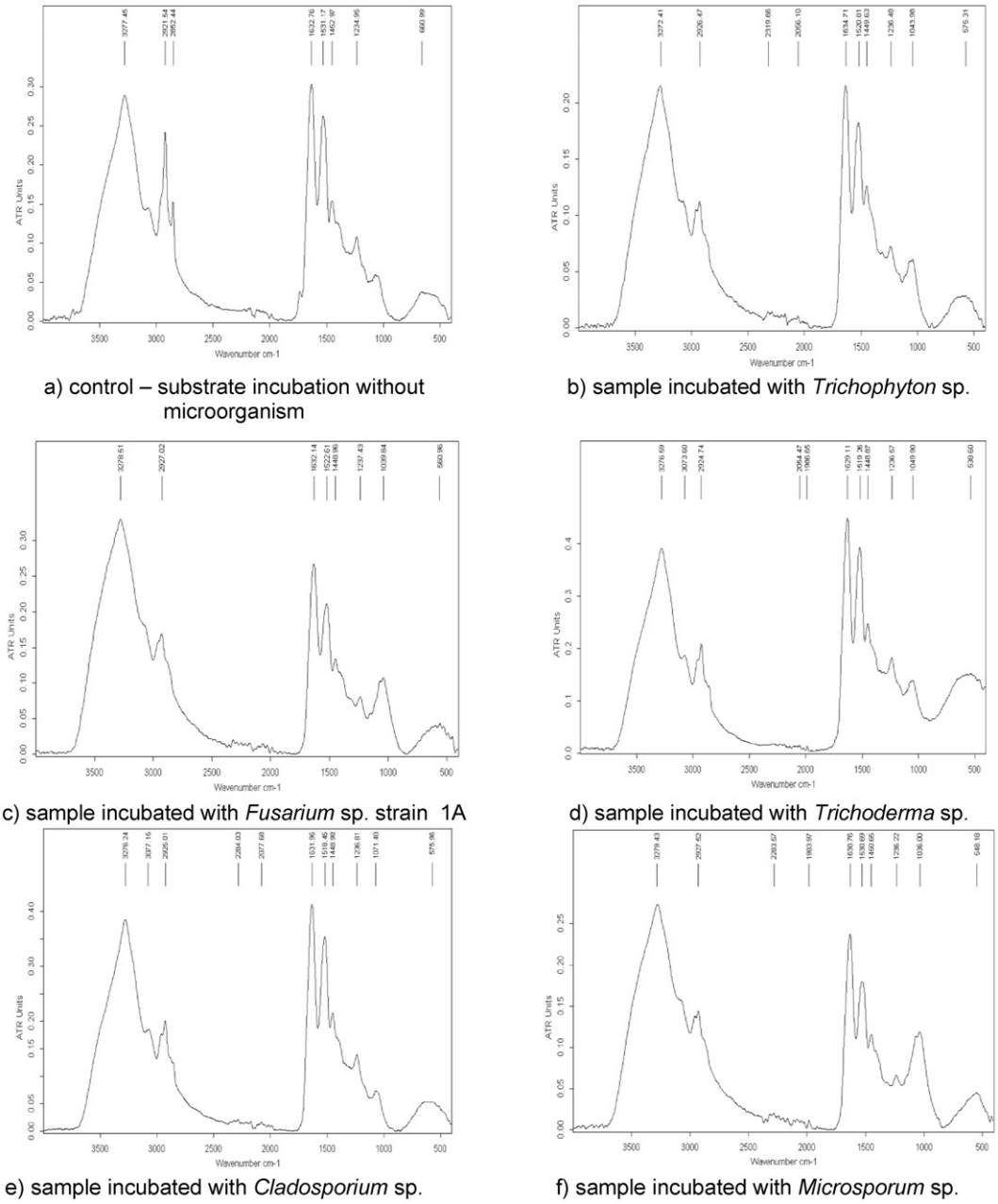


Fig. 2. FTIR spectra of horse hair strand incubated with various keratinolytic fungal strains.

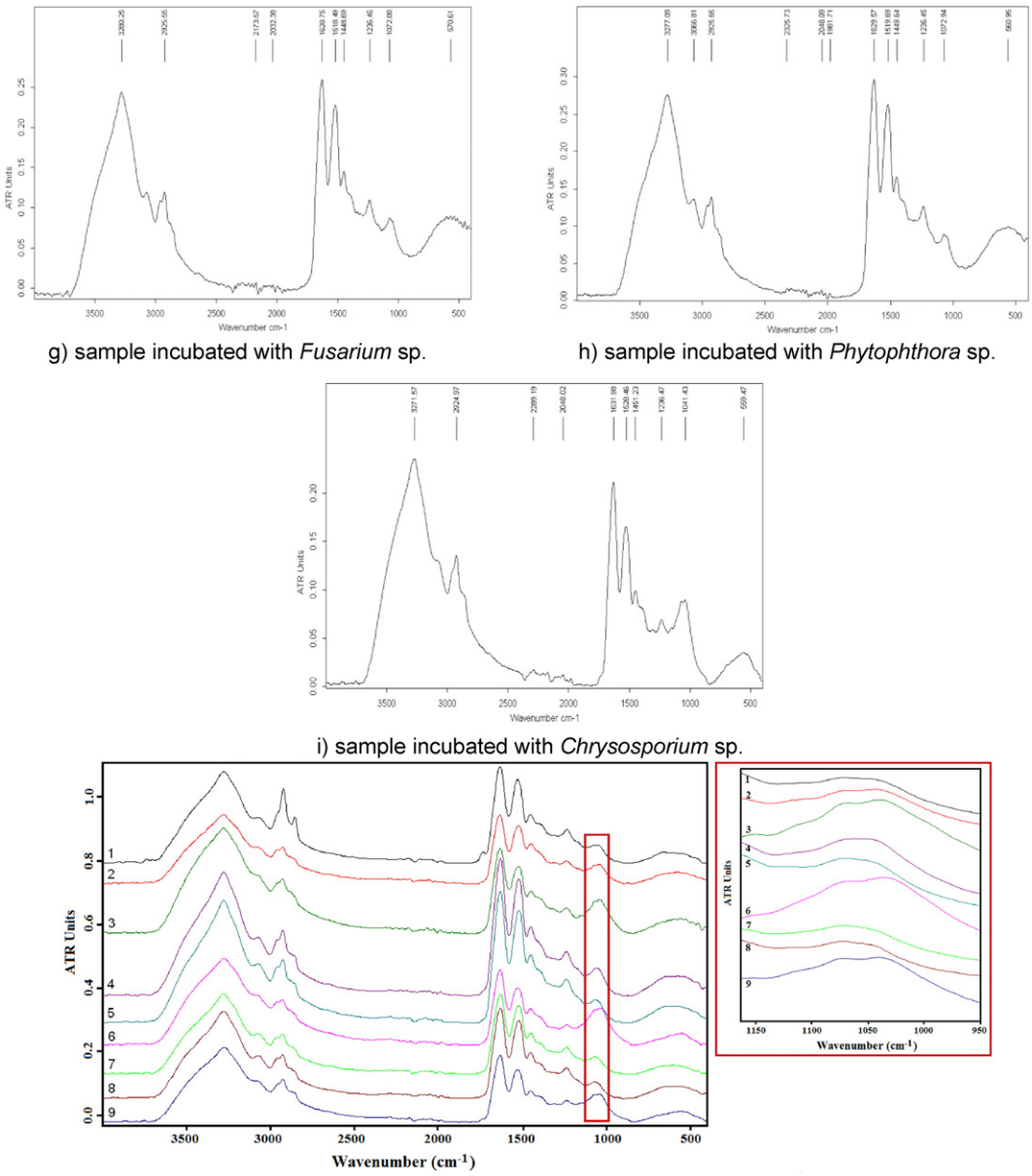


Fig. 2 (*continued*).

with untreated sample. In the case of samples with three degradation stages, the third stage occurred around 319–324°C. Two degradation stages, between 220°C and about 450°C, with maxims at 250–290°C, and around 326°C, were also observed in the degradation of keratin from human hair. Several degradation compounds were identified: ammonia, CO2, sulfur-containing inorganic compounds (SCS, SCO, and H2S), water, thiols, nitriles, phenol, and 4-methylphenol compounds [40].

Table 1
Weight loss of keratins materials during exposure to increasing temperatures.

Cod sample/TGA	Tested strain	Temperature domain (°C)/weight loss (%)			Residue (%)	Total weight loss (%)
		0–150°C	150–500°C	500–700°C		
1	Control (keratin virgin)	7.38	65.31	4.86	22.46	77.55
2	*Trichophyton* sp.	7.60	66.50	4.61	21.22	78.71
3	*Fusarium* sp. strain *1A*	7.14	71.10	4.21	17.51	82.45
4	*Trichoderma* sp.	7.75	68.27	4.46	19.44	80.48
5	*Cladosporium*	7.63	68.06	4.35	19.92	80.04
6	*Microsporum* sp.	7.42	63.69	4.41	23.53	75.52
7	*Fusarium* sp.	7.46	66.61	5.01	20.85	79.08
8	*Phytophthora* sp.	7.36	66.51	6.95	19.35	80.82
9	*Chrysosporium* sp.	8.02	63.01	3.70	25.28	74.73

Different amounts of residue were obtained after heating the samples at 700°C. Because the maximum amount of keratin residue (25.28%) was obtained in culture with *Chrysosporium* sp., it can be concluded that this strain was less active in keratin degradation. *Fusarium* sp. strain 1A was the most active in the degradation process, producing the strongest denaturation of polypeptide chains from a substrate weakened by fungal contact. This feature was evidenced by the production of the lowest amount of residue (17.51%) after heating at 700°C.

4. Conclusions

The current work asses the dynamics of keratin biodegradation by several keratinophilic fungal strains isolated from soil. The activity of fungal strains to degrade horse hair keratin was evaluated by SEM, FTIR spectra, and TGA analysis.

SEM micrographs offered detailed information on the interactions between microorganism and structural elements of hair strand. One may notice that all the tested strains could develop adequate structures related to keratin biodegradation, such as sleeve-like hyphae surrounding the strand, hyphae network and mycelia attached to the hair surface, and hyphae penetrating the cortex below cuticular scales.

The initiation of keratin biodegradation was demonstrated by FTIR by the bands at 1035–1075 cm^{-1} assigned to sulfoxide bond, which appeared as a result of S—S bond breaking. The highest peak areas were recorded for samples incubated with *Fusarium* sp. strain 1A, *Microsporum* sp. and *Chrysosporium* sp.

The TGA indicated the presence of three main zones of weight losses in samples; this thermal behavior was similar to the data reported in other papers. The highest weight loss was achieved in the second zone of thermal denaturation, where keratin suffers organic degradation of microfibrils and matrix. It was 71.10% weight loss for sample incubated with *Fusarium* sp. strain 1A. *Fusarium* sp. strain 1A was the most active organism in the degradation process, producing the strongest denaturation of polypeptide chains from a substrate weakened by fungal contact. This feature was evidenced by the production of the lowest amount of residue (17.51%) after heating at 700°C.

Because keratinolytic microorganisms and their enzymes keratinases are a subject of scientific and economic interest because of

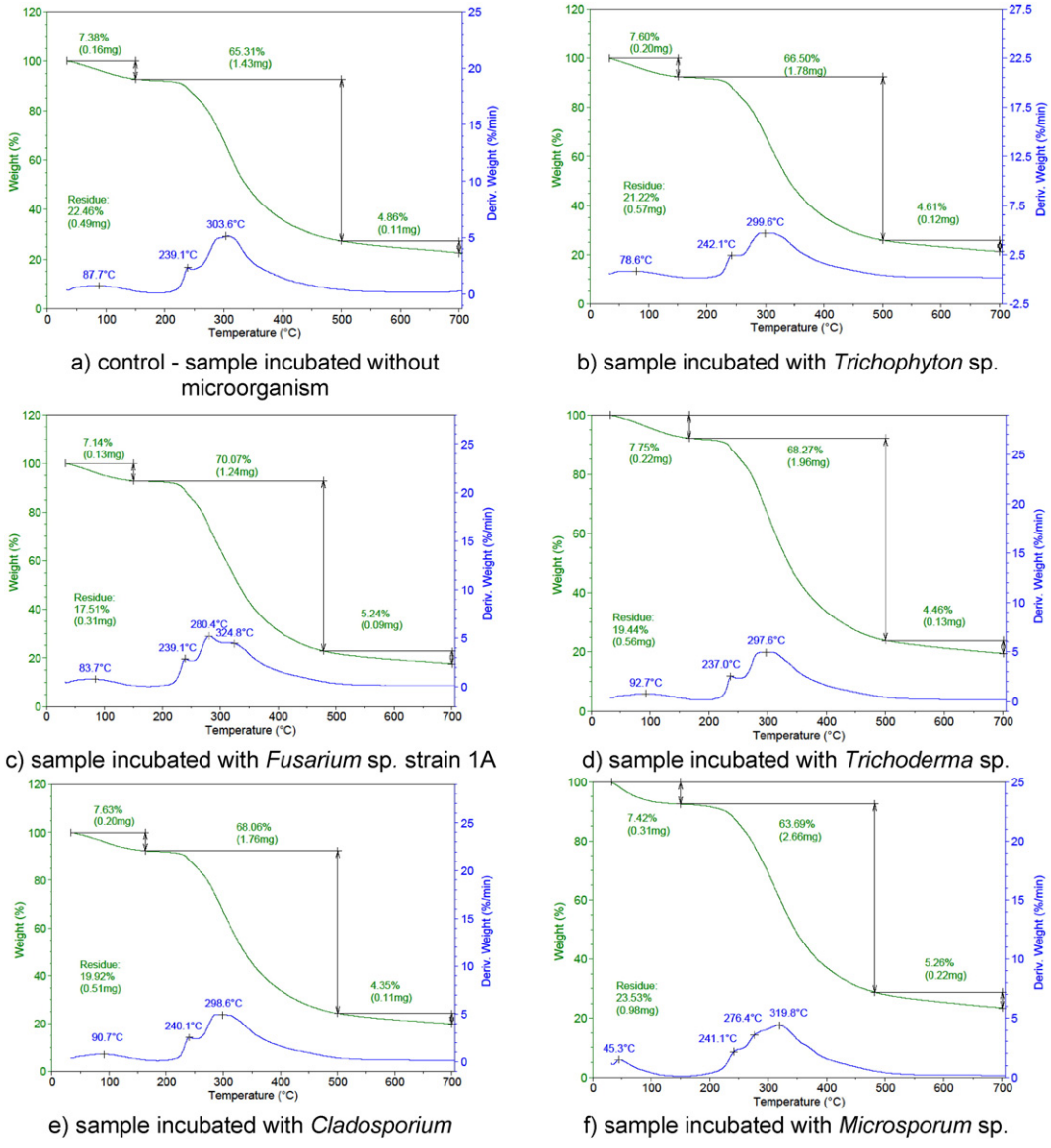


Fig. 3. TGA curves of horse hair strand incubated with keratinolytic fungi.

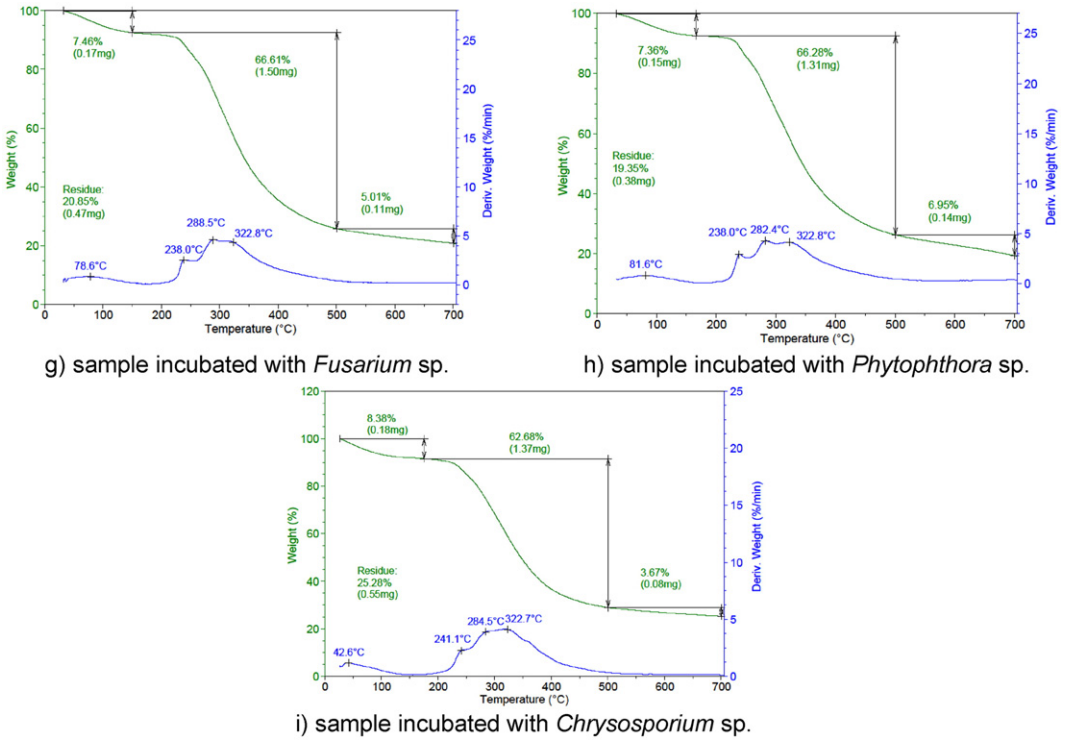


Fig. 3 (*continued*).

their capability to hydrolyze keratin, *Fusarium* sp. 1A, which has potential in the biotechnological process of biodegrading keratin, was selected for further studies.

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References

[1] Kreplak L, Doucet J, Dumas P, Briki F. New aspects of the α-helix to β-sheet transition in stretched hard α-keratin fibers. Biophys J 2004;87:640–7. http://dx.doi.org/10.1529/biophysj.103.036749.
[2] Bragulla HH, Homberger DG. Structure and functions of keratin proteins in simple, stratified, keratinized and cornified epithelia. J Anat 2009;214:516–59. http://dx.doi.org/10.1111/j.1469-7580.2009.01066.x.
[3] Lange L, Huang Y, Busk PK. Microbial decomposition of keratin in nature — A new hypothesis of industrial relevance. Appl Microbiol Biotechnol 2016;100:2083–96. http://dx.doi.org/10.1007/s00253-015-7262-1.
[4] Jin H-S, Park SY, Kim K, Lee Y-J, Nam G-W, Kang NJ, et al. Development of a keratinase activity assay using recombinant chicken feather keratin substrates. PLoS One 2017;12:e0172712. http://dx.doi.org/10.1371/journal.pone.0172712.
[5] Gupta R, Ramnani P. Microbial keratinases and their prospective applications: An overview. Appl Microbiol Biotechnol 2006;70:21–33. http://dx.doi.org/10.1007/s00253-005-0239-8.
[6] Jaouadi B, Abdelmalek B, Jaouadi Zaraî, Bejar NS. The bioengineering and industrial applications of bacterial alkaline proteases: The case of SAPB and KERAB. In: Carpi Angelo, editor. Progress in molecular and environmental bioengineering — from analysis and modeling to technology applications. InTech; 2011. p. 445–66.
[7] Brandelli A. Bacterial keratinases: Useful enzymes for bioprocessing agroindustrial wastes and beyond. Food Bioproc Tech 2008;1:105–16. http://dx.doi.org/10.1007/s11947-007-0025-y.
[8] Gopinath SCB, Periasamy A, Lakshmipriya T, Tang TH, Chen Yeng, et al. Biotechnological aspects and perspective of microbial keratinase production. Biomed Res Int 2015:140726. Hindawi Publishing Corporation, http://dx.doi.org/10.1155/2015/140726.
[9] Daroit DJ, Brandelli A. A current assessment on the production of bacterial keratinases. Crit Rev Biotechnol 2014;34:372–84. http://dx.doi.org/10.3109/07388551.2013.794768.
[10] Verma A, Singh H, Anwar S, Chattopadhyay A, Tiwari KK, Kaur S, et al. Microbial keratinases: Industrial enzymes with waste management potential. Crit Rev Biotechnol 2016;37:476–91. http://dx.doi.org/10.1080/07388551.2016.1185388.
[11] Liu B, Zhang J, Li B, Liao X, Du G, Chen J. Expression and characterization of extreme alkaline, oxidation-resistant keratinase from *Bacillus licheniformis* in recombinant *Bacillus subtilis* WB600 expression system and its application in wool fiber processing. World J Microbiol Biotechnol 2013;29:825–32. http://dx.doi.org/10.1007/s11274-012-1237-5.
[12] Ni H, Chen Q, Chen F, Ml Fu, Dong YC, Cai HN. Improved keratinase production for feather degradation by *Bacillus licheniformis* ZJUEL31410 in submerged cultivation. Afr J Biotechnol 2011;10:7236–44.
[13] Okoroma EA, Garelick H, Abiola OO, Purchase D. Identification and characterisation of a *Bacillus licheniformis* strain with profound keratinase activity for degradation of melanised feather. Int Biodeter Biodegr 2012;74:54–60. http://dx.doi.org/10.1016/j.ibiod.2012.07.013.
[14] e Silva LAD, Macedo AJ, Termignoni C. Production of keratinase by *Bacillus subtilis* S14. Ann Microbiol 2014;64:1725–33. http://dx.doi.org/10.1007/s13213-014-0816-0.
[15] Gröhs Ferrareze PA, Folmer Correa AP, Brandelli A. Purification and characterization of a keratinolytic protease produced by probiotic *Bacillus subtilis*. Biocatal Agric Biotechnol 2016;7:102–9. http://dx.doi.org/10.1016/j.bcab.2016.05.00.
[16] Cedrola SML, Melo ACN, Mazotto AM, Lins U, Zingali RB, Rosado AS, et al. Keratinases and sulfide from *Bacillus subtilis* SLC to recycle feather waste. World J Microbiol Biotechnol 2012;28:1259–69. http://dx.doi.org/10.1007/s11274-011-0930-0.
[17] Hong SJ, Park GS, Jung BK, Khan AR, Park YJ, Lee CH, et al. Isolation, identification, and characterization of a keratin-degrading bacterium *Chryseobacterium* sp. P1-3. J Appl Biol Chem 2015;58:247–51. http://dx.doi.org/10.3839/jabc.2015.039.
[18] Riffel A, Brandelli A, Bellato CM, Souza GHMF, Eberlin MN, Tavares FCA. Purification and characterization of a keratinolytic metalloprotease from *Chryseobacterium sp.* kr6. J Biotechnol 2007;128:693–703. http://dx.doi.org/10.1016/j.jbiotec.2006.11.007.
[19] Gurav RG, Jadhav JP. Biodegradation of keratinous waste by *Chryseobacterium* sp. RBT isolated from soil contaminated with poultry waste. J Basic Microbiol 2012;53:128–35. http://dx.doi.org/10.1002/jobm.201100371.
[20] Allure N, Madhusudhan DN, Agsar D. Enhanced production, purification and characterization of alkaline keratinase from *Streptomyces minutiscleroticus* DNA38. Int Lett Nat Sci 2015;43:27–37. http://dx.doi.org/10.18052/www.scipress.com/ILNS.43.27.
[21] Syed DG, Lee JC, Li WJ, Kim CJ, Agasar D. Production, characterization and application of keratinase from *Streptomyces gulbargensis*. Bioresour Technol 2009;100:1868–71. http://dx.doi.org/10.1016/j.biortech.2009.08.026.
[22] Lopes FC, Dedavid e Silva LA, Tichota DM, Daroit DJ, Velho RV, Pereira JQ, et al. Production of proteolytic enzymes by a keratin-degrading *Aspergillus niger*. Enzym Res 2011. http://dx.doi.org/10.4061/2011/487093.

[23] Mazotto AM, Couri S, Damaso MCT, Vermelho AB. Degradation of feather waste by *Aspergillus niger* keratinases: Comparison of submerged and solid-state fermentation. Int Biodeter Biodegr 2013;85:189–95. http://dx.doi.org/10.1016/j.ibiod.2013.07.003.
[24] Nwadiaro PO, Chuku A, Onyimba IA, Ogbonna AI, Nwaukwu IA, Adekojo DA. Keratin degradation by *Penicillium purpurogenum* isolated from tannery soils in Jos, Nigeria. Br Microbiol Res J 2015;8:358–66. http://dx.doi.org/10.9734/BMRJ/2015/16339.
[25] El-Gendy MMA. Keratinase production by endophytic *Penicillium* spp. Morsy1 under solid-state fermentation using rice straw. Appl Biochem Biotechnol 2010;162: 780–94. http://dx.doi.org/10.1016/S0141-0229(02)00283-1.
[26] Kannahi M, Ancy RJ. Keratin degradation and enzyme producing ability of *Aspergillus flavus* and *Fusarium solani* from soil. J Chem Pharm Res 2012;4:3245–8. http://dx.doi.org/10.1139/w06-067.
[27] Sowjanya NC, Chary CM. Degradation of few avean feathers by *Microsporum gypseum*. J Phytology 2012;4:21–3.
[28] Cao L, Tan H, Liu Y, Xue X, Zhou S. Characterization of a new keratinolytic *Trichoderma atroviride* strain F6 that completely degrades native chicken feather. Lett Appl Microbiol 2008;46:389–94. http://dx.doi.org/10.1111/j.1472-765X.2008.02327.x.
[29] Maruthi AY, Lakshmi AK, Rao RS, Chaitanya AD. Degradation of feather and hair by *Chrysosporium tropicum*: A potent keratinophilic fungus. Afr J Biotechnol 2011;10: 3579–84. http://dx.doi.org/10.5897/AJB10.432.
[30] Bohacz J. Biodegradation of feather waste keratin by a keratinolytic soil fungus of the genus *Chrysosporium* and statistical optimization of feather mass loss. World J Microbiol Biotechnol 2017;33:13. http://dx.doi.org/10.1007/s11274-016-2177-2.
[31] Blyskal B. Fungi utilizing keratinous substrates. Int Biodeter Biodegr 2009;63: 631–53. http://dx.doi.org/10.1016/j.ibiod.2009.02.006.
[32] Yang FC, Zhang Y, Rheinstädter MC. The structure of people's hair. Peer J 2014; 2:e619. http://dx.doi.org/10.7717/peerj.619.
[33] Marchisio VF, Fusconi A, Rigo S. Keratinolysis and its morphological expression in hair digestion by airborne fungi. Mycopathologia 1994;127:103–15. http://dx.doi.org/10.1007/BF01103066.
[34] Marchisio VF. Keratinophilic fungi: Their role in nature and degradation of keratinic substrates. In: Kushwaha RKS, Guarro J, editors. Biology of dermatophytes and other keratinophilic fungi. Bilbao, Spain: Rev Iberoam Micol; 2000. p. 86–92. [ISBN: 84-607-0711-3].
[35] Vasconcelos A, Freddi G, Cavaco PA. Biodegradable materials based on silk fibroin and keratin. Biomacromolecules 2008;9:1299–305. http://dx.doi.org/10.1021/bm7012789.
[36] Cardamone JM, Nunez A, Garcia RA, Ramos MA. Characterizing wool keratin. Res Lett Mater Sci 2009;2009:147175. 5 pp. http://dx.doi.org/10.1155/2009/147175.
[37] Broda J, Przybyło S, Kobiela-Mendrek K, Binia D, Rom M, et al. Biodegradation of sheep wool geotextiles. Int Biodeter Biodegr 2016;115:31–8. http://dx.doi.org/10.1016/j.ibiod.2016.07.012.
[38] Istrate D, Popescu C, Rafik ME, Möller M. The effect of pH on the thermal stability of fibrous hard alpha-keratins. Polym Degrad Stab 2013;98:542–9. http://dx.doi.org/10.1016/j.polymdegradstab.2012.12.001.
[39] Liu X, Gu S, Xu W. Thermal and structural characterization of superfine down powder. J Therm Anal Calorim 2013;111:259–66. http://dx.doi.org/10.1007/s10973-012-2202-0.
[40] Brebu M, Simion I. Thermal degradation of keratin waste. J Anal Appl Pyrolysis 2011; 91:288–95. http://dx.doi.org/10.1016/j.jaap.2011.03.003.