Electronic Journal of Biotechnology 28 (2017) 101–112

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Research article

Degradation of keratin substrates by keratinolytic fungi

Mariana Călin [a,b], Diana Constantinescu-Aruxandei [a], Elvira Alexandrescu [a], Iuliana Răut [a], Mihaela Badea Doni [a], Melania-Liliana Arsene [a], Florin Oancea [a], Luiza Jecu [a,*], Veronica Lazăr [b]

[a] Biotechnology Department, National Institute for Research & Development in Chemistry and Petrochemistry-ICECHIM, 202 Independentei Spl., 060021, Bucharest, Romania
[b] Faculty of Biology, University of Bucharest, 91–95 Independentei Spl., Bucharest, Romania

ARTICLE INFO

Article history:
Received 28 February 2017
Accepted 24 May 2017
Available online 30 May 2017

Keywords:
Biodegradation
FTIR
Fungal species
Fusarium
Keratin degradation
Keratinases
Keratinolytic fungi
SEM
TGA

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

* Corresponding author.
E-mail address: jecu.luiza@icechim.ro (L. Jecu).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

http://dx.doi.org/10.1016/j.ejbt.2017.05.007

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.

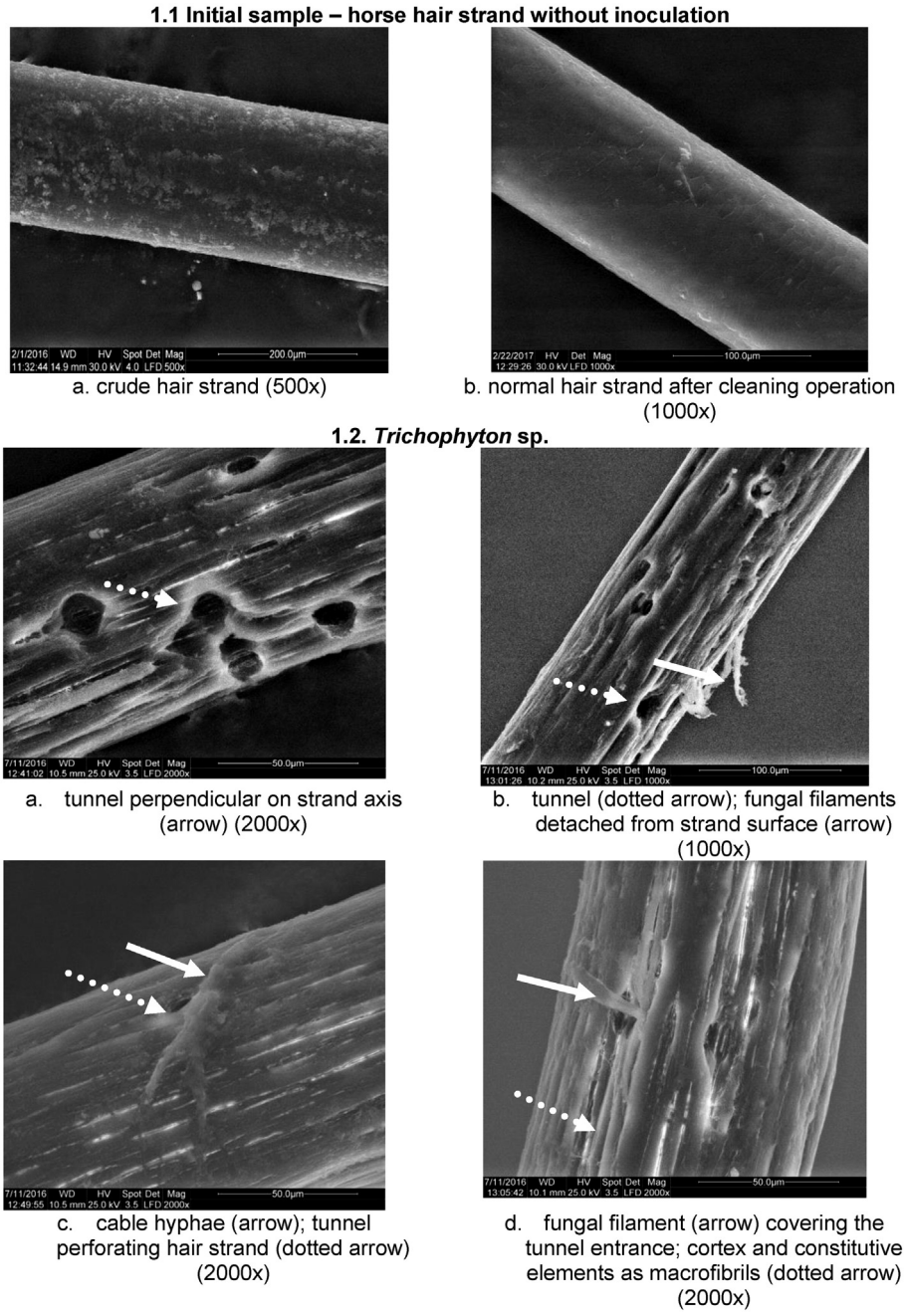

Fig. 1. SEM images of horse hair strands incubated with keratinolytic fungi grown on agitated liquid cultures (micrographs of 21-d culture). 1.1. Initial sample without inoculation; 1.2. *Trichophyton* sp.; 1.3. *Fusarium* sp. strain 1A; 1.4. *Trichoderma* sp.; 1.5.*Cladosporium* sp.; 1.6. *Microsporum*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