Tools to compare enzymatic unhairing action of two fungal strains

B. Galarza[1*], L. Garro[1], N. Ferreri[2], L. Elíades[2]

[1]Research Center for Leather Technology (CITEC), CICPBA, Camino Centenario y 508, (1897) Gonnet, Argentina
[2] Institute of Botany Carlos Spegazzini, CONICET, FCNM, UNLP, 53 n°477 (B190BAJ), La Plata, Argentina
*betinagal@hotmail.com

Introduction

Enzymatic unhairing represent a paradigm shift in leather technology and it's a environmentally friendly. Fungal enzymatic extracts with keratinolytic activity can be obtained from solid substrate fermentation using hair waste from saving unhairing as a source of C, N and energy.

Objectives

- To compare the depilatory action and morphological changes on bovine skin of fungal enzyme extracts of *Fusarium oxysporum* and *Trichophyton ajelloi* through *in vitro* and *in vivo* keratinolytic activity assays.
- To provide analytical tools to predict the action of enzyme extracts on bovine skin.

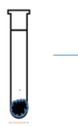
Materials and methods

Fusarium oxysporum (FO) strain, a saprotrophic fungus and opportunistic pathogen was isolated from alcaline soils of coast of Buenos Aires Province. *Trichophyton ajelloi* (TA), an exceptionally pathogenic dermatophyte were previously isolated and identified by the method of hair´s baiting of Vanbreuseghem (Deschmukh *et al.*, 2012).

Fungal enzymatic extract (EE) were obtained by solid substrate fermentation using hair waste (HW) from hair-saving unhairing process as a substrate (Galarza *et al.*, 2019).

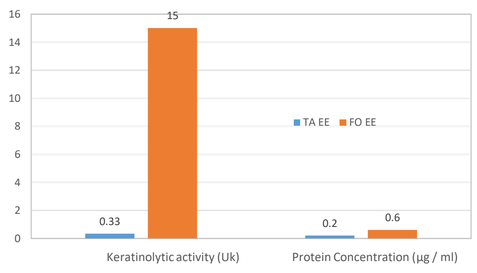
Keratinolytic activity *in vitro*

(**KAIVT**)*(Uk: EE absorbance increase of 0,01 $Abs_{280\ nm}$ / min under test condition condition)*



HW 1% in Buffer Tris-HCl 0,1 M pH+ 150 µl EE

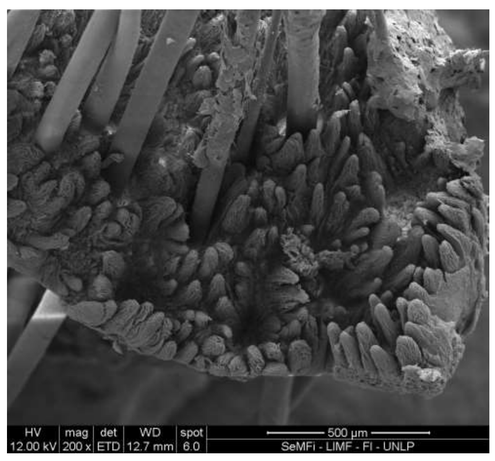
Incubation:37°C-100rpm-60 min
Stop: 1ml of TCA 10% (w/v)
Centrifugation 5000g 15min
OD 280nm (triplicate)
Reaction blanks: EE inactivated with TCA 10% (w/v) at the beginning of the incubation with HW



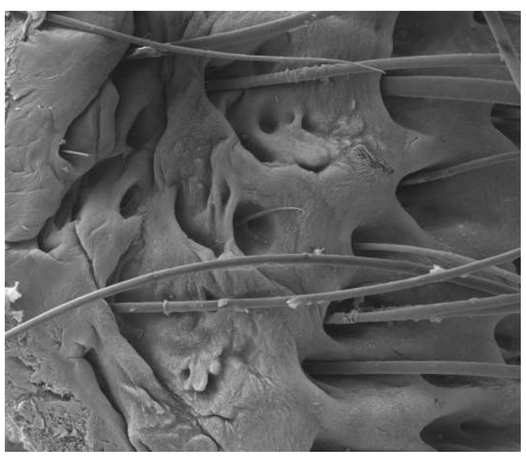
Variation in Keratinolytic Activity (left) and Protein Concentration (Bradford's Method, 1976) (right) from ***Trichophyton ajelloi*** (TA) and ***Fusarium oxysporum*** (FO) enzymatic extracts (EE)

Keratinolytic activity *in vivo* (KAIV)

Laboratory scale: in flat-bottom reaction tubes of 60 ml pieces of bovine skin immersed with: EE (1:1), biocide TCMTB 0.2% anionic tensioactive 0.1% (4 hs), tensioactive non-ionic 0, 5% (unhairing step). Control test in same condition whitout EE. Incubation 48 h 37°C 40 rpm.
Morphological changes were observed by Scanning Electron Microscopy (SEM)



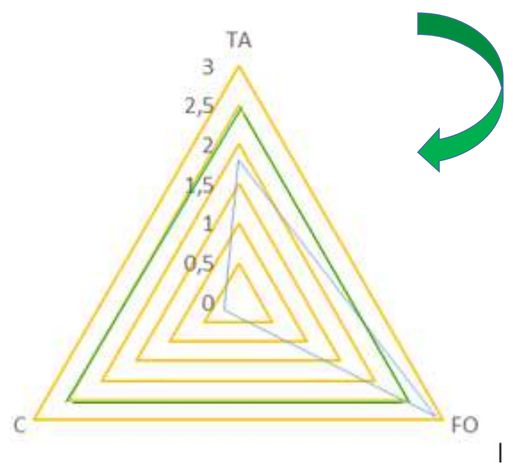
KAIV: *Fusarium oxysporum* EE action: visible dermal papilla, absence of epidermis, detachment of hair follicle sheath (SEM 400x)



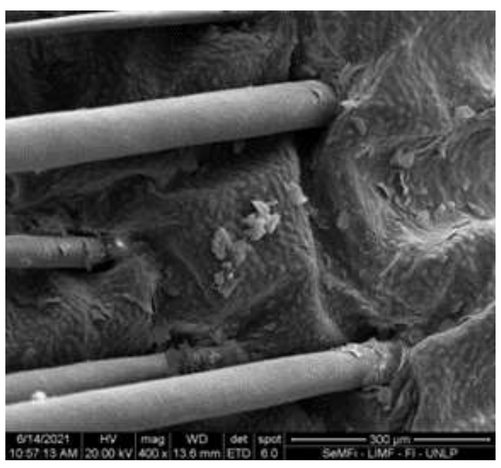
KAIV: *Trichophyton ajelloi* EE action: absence of epidermis, detachment of hair follicle sheath,, empty hair follicles (SEM 300 x)

From the observations with SEM, a score (from 0 to +3, in increasing order of histological action) was defined according to the following parameters of morphological changes:

- hair, epidermis with normal patterns (NP)
- detachment of hair follicle sheath (DHFS)
- absence of epidermis (AE)
- empty hair follicles (EHF)
- visible dermal papilla (VDP)



Spider Plot of the parameter score: The green line 2,5 shows the optimal values into account the changes in the epidermis, dermis and hair follicle. TA EE is below the optimal value and FO EE is above



TA EE with lower KAIVT showed fewer morphological changes (KAIV) than the FO EE

Control C: hair, epidermis with normal patterns (SEM 400x)

Conclusions

A linear correlation can be established between the keratinolytic activity *in vitro* (KAIVT) and *in vivo* (KAIV). The greater *in vitro* activity corresponds to a greater *in vivo* activity at the level of histological changes.
These laboratory tools can predict enzyme action in a simple and low-cost way to evaluate EE products of biological origin.

References
Bradford, M., (1976). *Analitycal Biochemistry*, 72, 248.
Deschmukh, S., Verekar, S., (2012). *Microbiology Research*, 3(1), 24-27.
Garro L., Galarza B., Greco C., Hours R. (2019). *Journal of the Society of Leather Technologists and Chemists*, 103 (1), 28-34