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LEATHER HYDROLYSATE EVALUATED AS ORGANIC NITROGEN SOIL INPUT

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Abstract

The leather manufacturing process produces annual tons of misspends being considered very harmful for nature. Solid waste generated in leather industry contains protein as main component. The biotechnological sector allows us to use this worthless material as microbial substrate for enzyme production. Those enzymes have multiple uses and can cover numerous industrial needs.
This paper covers some potential applications in the agriculture sector as fertilizer. Leather hydrolysate results from bacterial conversion of leather proteins. The specific bacteria where obtained through isolation of the compostation of leather and incubated 48 hours into a minimal media. During the optimization process the maximum proteinase production was 1.5 U/ml and it was achieved after 120 hours of incubation at 35°C, using a minimal media and 0.6 shredded leather. Leather debris containing proteins and amino acids which can be applied as bio-growth increase by environmental secure technologies, and eventually in organic output production.
The leather hydrolysate resulting from the microbial conversion of hide protein can be manipulated as organic nitrogen soil input.

Key words: *leather degradation, bacterial isolation, proteinase, fertilizer.*

INTRODUCTION

Low commercial value proteinase waste of animal origin such as skins and fur are generated in huge amount in skins and fur industry and are hard-to-degrade. Currently this waste is disposed by incineration which can lead to serious pollution of the environment.

An alternative to incineration is the microbial degradation, through this process obtaining proteinase enzyme, which can be applied as agricultural fertilizer, being rich in nitrogen and also inexpensive.

MATERIALS AND METHODS

Three isolated bacteria from compostation on fur and skins were used on 3000 mL minimal media (MM)(g/L - 1.0g $NaCl_2$, 0.05g $CaCl_2$, 0.7g KH_2PO_4, 0.9g $MgSO_4$, 2.38g K_2HPO_4, 3,0g sucrose, 0.6g skin and fur, pH 7,2), sterilized at 121°C, 20 min, incubated at 35°C, 135 rpm, 120 hours.

ISOLATION OF PROTEIN HYDROLYZING BACTERIUM

Skins and fur degrading bacteria was isolated from compostation of skin and fur soil for 90 days.

Upon incubation 1cm^3 of soil was prepared in suspension in 40 mL minimal media for 48 h, at 35^{o}C and 135 rpm. After incubation the isolated colonies were spread on PCA plates and incubated 24 h at 35^{o}C.

Individual colonies exhibit clear halo around them, resulting from proteinase hydrolysis, were picked and purified by repeated sub-culturing on Luria Bertani agar plates. The pure colonies were screened for their ability to degrade fur and skins on minimal media at 600nm (Habib et al.,2012)

PROFILE OF PROTEASES PRODUCTION BY ISOLATES

The 500 mL Erlenmeyer flasks containing 0.6% (w/v) skins and fur were inoculated with isolated cultured on MM to obtain an initial cultured density (OD_{600NM}). They were incubated 120 h at 35^{o}C, 135 rpm. The turbidity was measured at 24 h, 36 h, 48 h, 72 h, 96 h and 120 h of incubation and monitored for growth and protease activity.

CONCENTRATION OF ENZYMES

The fermentation broth was harvested after 120 hours of incubation and it was centrifuged at 4^{o}C and 9000xg for 20 minutes. The supernatant was collected and concentrated ten times using the rotary evaporator at 60^{o}C.

USE OF SKINS AND FUR AS BIOACTIVE AGRICULTURAL FERTILIZER (6 DAYS PLANT GROWTH)

The study was carried out in different concentration(1%;1,5%;2%;2,5%;3%;3,5%;10%; 15%;20%;25%;20%;35%) and in dilution of 1:10 mL in 100 mL plastic pots with 40 g of soil and 4 g of grain seeds (276 mL of skins hydrolysates/ 4320g soil)

All the pots were watered regularly with tap water to reduce the evaporation loss. After 6 days of sowing all plants were uprooted and washed. The growth parameters (plant height and root length) were recorded.

ANALYTICAL PROCEDURE: PROTEINASE ACTIVITY

Proteolytic activity was spectrophotometric measured at 578nm. The reaction mix contained 0.5mL enzymatic solution and 1 mL casein 1% in phosphate buffer 0.2M (pH 7), incubated at 37^{o}C for 10 min. Enzymatic reaction was stopped with 2 ml of trichloroacetic acid 5%. The reaction mix was kept 30 min at room's temperature and then it was filtrated. For every 0.5mL filtrate were added 0.5mL HCl 0.2N, 2mL NaOH 0.5N and 0.6 mL Folin-Ciocalteu 1:2. After 30 min at room's temperature the extinction was measured.

One unit of proteases activity is defined as the amount of enzyme that releases 1µmol tyrosine per minute, under analysis condition.

RESULTS AND DISCUSSIONS

ISOLATION OF PROTEOLYTIC HYDROLYZING BACTERIA

Three proteolytic bacteria strains (DA 7, DA 10 DA 13) isolated from the compotation of skins and fur were screened for skin degradation on PDA indicated their ability to use fur and skins protein as a sole carbon source for growth.

MEDIA COMPONENTS OPTIMIZATION FOR PROTEASE PRODUCTION

The isolates produced maximum proteolytic enzyme (1,213 U/mL) in the presence of 3 g of glucose and fur as substrate in a MM, a phenomenon observed for biosynthesis of bacterial proteases.

Table 1. Proteolytic screening of the isolated strains (DA 7, DA 10, DA 13) at 578 NM after 120 h incubation at 135rpm and 35°C.

Samples	Dilution	24 h	48 h	72 h	96 h	120 h	Media
DA 7	1:10	0.231	0.415	0.586	0.938	0.993	0.633
DA 10	1:10	0.556	0.630	0.815	0.906	0.987	0.779
DA 13	1:10	0.183	0.139	0.426	0.506	0.616	0.374

THE APPLICATION OF SKINS AND FUR FERMENTATION AS FERTILIZER IN AGRICULTURAL FIELD

The content of proteinase hydrolysates resulting from the conversion of fur and skins proteins by isolates was 1,213 U/mL.

Accordingly the skins and fur digest was concentrated 10 times and evaluated for its use as an organic fertilizer on the growth of *Hordeum vulgare L.* It was observed that the added skins hydrolysates exerted a beneficial effect on the germination. Plant height and root length was increased by 79, 86 and 73 % in the treated soil over the untreated soil. (Similar effect has been reported by Kim et al. (2005), A. Bose et al. (2013) and Vasileva-Tonkova et al. (2009) but on feather hydrolysate as nitrogen input)

The skins and fur hydrolysate obtained upon decomposition of skins and fur by isolates, holds potential be applied as agricultural fertilizer.

Figure 1 Wheat after 6 days of growth, concentration 35% against the blank

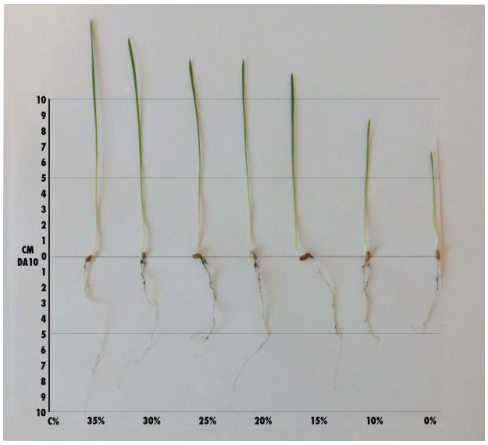

Figure 2 Wheat fertilized with DA10 strain after 6 days of growth at concentrations varying between 35% (left) and 10% (right)

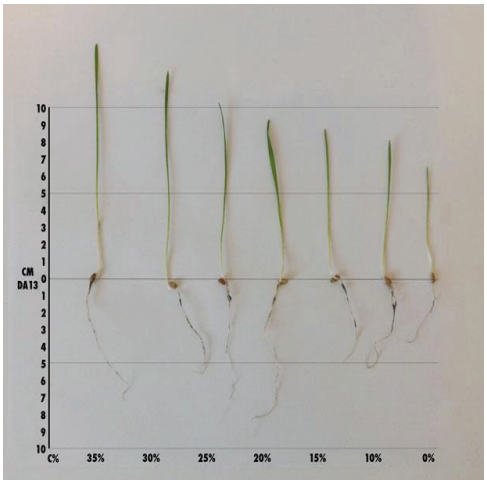

Figure 3 DA13 strain at different concentration between 35% (left) and 10% (right), after 6 days of growth. On the right we have the blank for comparison.

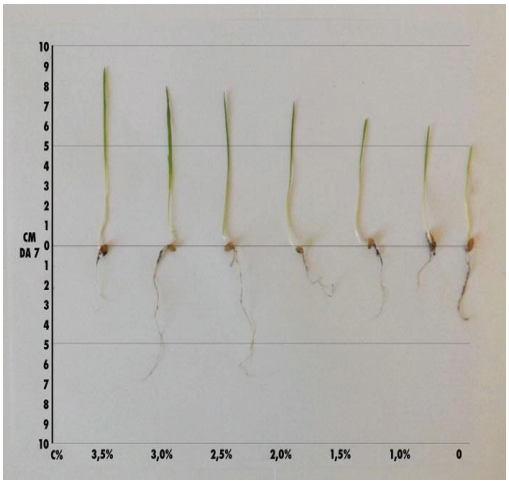

Figure 4 The wheat after 6 days of growth, after adding concentrated enzyme produced by DA7 strain, 3,5% to 1,5 % concentration from left to right against the blank

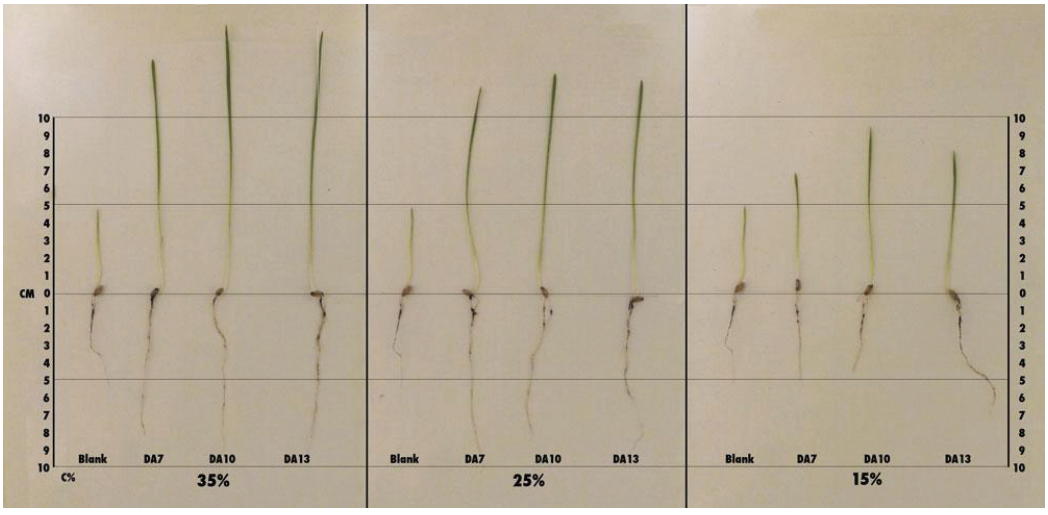

Figure 5 Compared wheat growth after 6 days with added concentrated enzymatic solution (35%, 25%, 15% from left to right) produced by the 3 isolated strains DA7, DA10, DA13 against the blank.

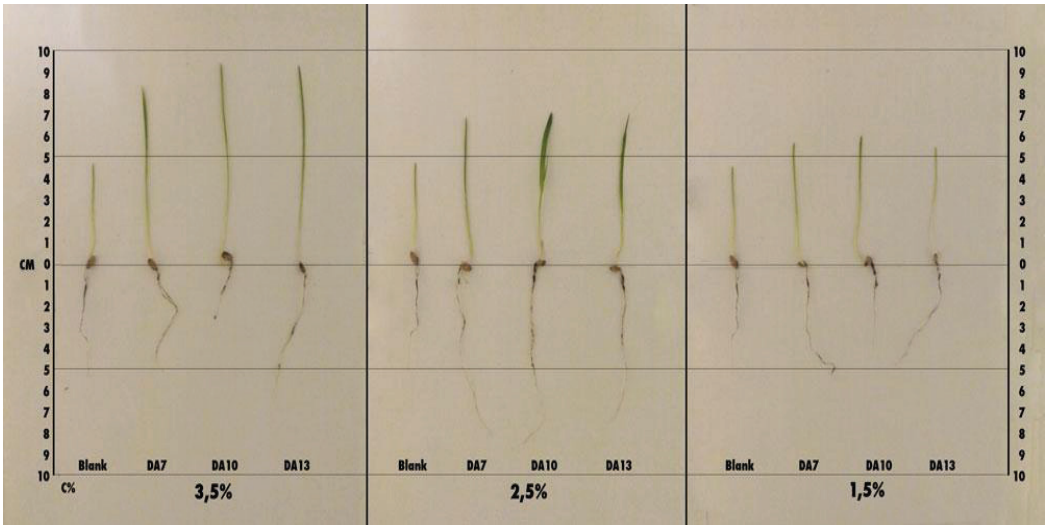

Figure 6 The wheat growth after 6 days at different enzymatic concentrations (3,5%, 2,5%,1,5%, from left to right) of the 3 isolated strains DA7, DA10, DA13 compared with the blank

CONCLUSIONS

In the present study the strains isolated from the compostation of the skins and fur were found to efficiently degrade fur in 120 h of incubation, at 135 rpm, 35°C. The strains ability to degrade makes it a potential tool for the development of suitable processes for conversion of fur and skins into fertilizer.

The best growth was obtained using 35% enzymatic concentration produced by DA10 strain.

The enzyme production was characterized and used as fertilizer soil input which proved from the evaluation as beneficial for plant growth.

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