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LEATHER HYDOLYSATE EVALUATED AS BIOACTIVE POTATO FERTILIZER

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Abstract
Leather industry discharges enormous amount of chrome containing leather solid wasted which creates a major disposal problem. Tanned leather solid waste is a complex of hard-to-degrade proteins and chromium. The biotechnological sector allows us to use the waste materials as bacterial substrate for enzyme production. The present work covers potential application in the potato bio-growth as fertilizer. The hydrolysate results from bacterial conversion of leather components. Bacteria was isolated from the composting of leather and incubated into a minimal media for 120 hours at 35°C. In the optimization process maximum proteinase production was 1.223 U/ml. The results obtained suggested that leather debris containing amino-acids and proteins andcan be applied as organic nitrogen soil input.

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

INTRODUCTION

Proteolytic enzymes are involved in breakage of the long chain molecules of proteins into shorter fragments – peptides and eventually into their components, amino-acids (Anson, 1938).
Low commercial value protein waste of animal origin such as skins and the manufacturing processes of leather produce annual tones of worthless material. Through the biotechnological methods this waste materials can be used for obtaining hydrolytic enzymes, which can be applied as agricultural fertilizer.
Nitrogen management is perhaps the most important aspect of successful potato (*Solanum tuberosum* L.) production.
The high cost of chemical fertilizer along with the related ecological and health hazards necessitate finding out an alternative nutrient sources to sustain the crop yield without any adverse effect on soil and environment. The aim of this work was the isolation of bacterial strains able to produce proteases involved in leather degradation and the use of the concentrated fermentation liquids as plant fertilizer.

MATERIALS AND METHODS

The strains used in experiments were isolated from composting of chromium tanned leather (leather debris were included in soil samples and incubated at room temperature for 3 months), according to classical microbiological methods.
The leather debris was kindly provided by the National Research and Development Institute for Textiles and Leather Bucharest.
The bacteria were screened for their ability to degrade proteins on selective medium (Habib et al., 2012).
The selected bacteria were grown in minimal media (MM) (g/L - 1.0g $NaCl_2$, 0.05 g $CaCl_2$, 0.7 g KH_2PO_4, 0.9 g $MgSO_4$, 2.38 g K_2HPO_4, 3,0 g sucrose, with 0.6 g leather as nitrogen source, pH 7,2) (Israel et al., 2012).
After inoculation, the bacterial fermentation was performed at 35°C for 120 hours, with stirring (135 rpm). The turbidity (OD600 nm) 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.

Biomass separation and concentration of enzymes

At the end of fermentation process the cultivation medium was separated by centrifugation at 9000 rpm, at 4°C, for 20 minutes. The supernatant (fermentation liquid) was collected and concentrated ten times at 60°C, using the rotary evaporator.

The proteinase activity

Proteolytic activity was measured spectrophotometrically at 578 nm, following the method of Anson (1938). The reaction mix contained 0.5 mL enzymatic solution and 1 mL casein 1% in phosphate buffer 0.2M (pH 7), incubated at 37°C for 10 min. Enzymatic reaction was stopped with 2 mL oftrichloroaceticacid 5%. The reaction mix was kept 30 min at room's temperature and then it was filtrated. For every 0.5mL filtrate was added 0.5mL HCl 0.2N, 2mL NaOH 0.5N and 0.6 mL Folin-Ciocâlteu 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.

Testing leather hydrolysate as bioactive agricultural fertilizer

Liquid fermentation media obtained after biomass separation, named leather hydrolysate, was used as plant biofertilizer.

Different concentrations of leather hydrolysed (15%; 20%; 35%) were added in dilution of 1:10 mL in 100 mL plastic pots with 40 g of soil, and 4 week sold potato plants grown from the meristem were planted. Two potato varieties were used in experiments: *Solanum tuberosum* var. *Christian)* and *S. Tuberosum* var. *Roclas.* All the pots were watered regularly. After 6 days of sowing all plants were uprooted and washed. The growth parameters (plants length and number of leaves) were observed.

RESULTS AND DISCUSSIONS

Three bacterial strains designated DA7, DA10 and DA13 were isolated from the compost with chromium tanned leather. Their growth determined after the cultivation in minimal medium for 120h is presented in table 1.

Table 1. Growth efficiency (OD600nm) of the selected bacterial strains in minimal medium for 24-120 h

Samples	24 h	48 h	72 h	96 h	120h	Average
DA 7	0.239	0.381	0.487	0.699	0.917	0.544
DA 10	0.389	0.592	0.721	0.938	1.019	0.731
DA 13	0.244	0.390	0.515	0.725	0.997	0.574

The proteolytic activity of the selected strains was determined. The maximum enzymatic activity, 1.223 U/mL was obtained with the strains DA10, after 120h of cultivation in minimal medium with chromium tanned leather as nitrogen source.

The fermentation broth was concentrated 10 times and evaluated as biofertilizeron the growth of *Solanum tuberosum* L. plants. It was observed that the added leather hydrolysates exerted a beneficial effect on the plant growth. Plant and root length increased in the treated variants over the untreated ones. Similar effect has been reported by different authors (Kim et al., 2005; Bose et al., 2013;Vasileva-Tonkova et al., 2009) when feather hydrolysate was used as nitrogen supplement in soil.

The strain DA13 was less efficient, event at 35% concentration, the results being correlated with the enzymatic activity determined previously (Figure 1).

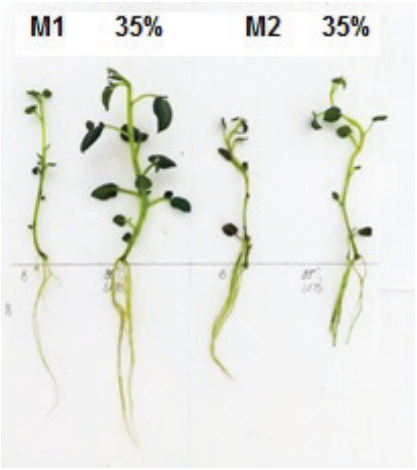

Figure 1. The efficiency of DA13 fermentation broth treatment at 35%.
M1 – Christian variety; M2 – Roclas variety

The best results were obtained with the concentrated broth from DA10 strain, for both potato varieties (Figure 2).

Among the concentrations used in treatments, the most efficient variant was 35% (Figure 3).

Differences between potato varieties were also observed: the highest plants were observed at Roclas variety, but the most vigorous were the plants from Christian variety.

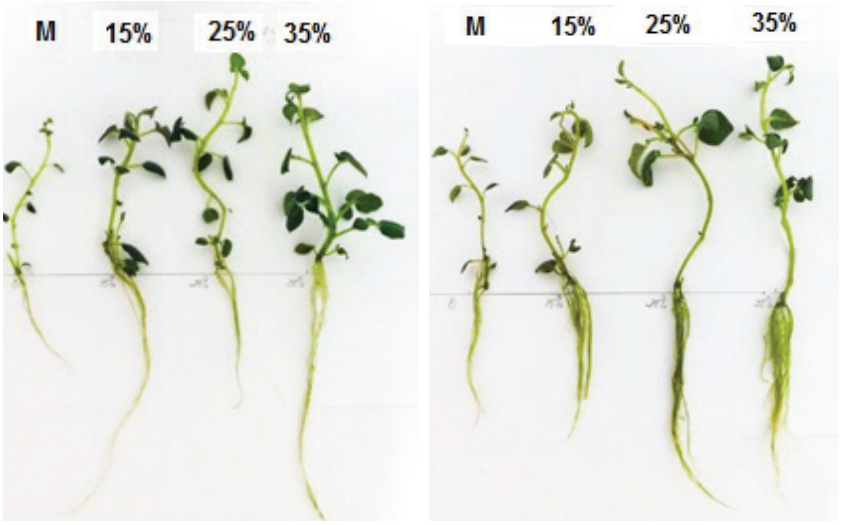

Figure 2. The effect of the application of the DA10 fermentation broth in different concentrations (15%, 25%, and 35%) on potato plants from Christian (left) and Roclas (right) varieties.

Figure 3. The efficiency of DA10 fermentation broth treatment at 35%. M1 – Christian variety; M2 – Roclas variety

CONCLUSIONS

Three new proteolytic bacterial strains were selected from compost (soil with leather debris composted during 3 months). Among them, the strain designated DA10 exhibited both the highest rate of growth and the best proteolytic activity in minimal medium with chromium tanned leather as nitrogen source.

The fermentation broths obtained with all the bacterial strains were used as biofertilizer for potato plants. Differences between bacterial treatments and potato varieties were observed: the best strain was DA10 in concentration of 35%, and the most vigorous were the plants from *Solanum tuberosum L*. Christian variety.
The results obtained demonstrate that the fermentation broths could contain hydrolytic enzymes able to liberate amino-acids that stimulate the plant growth.

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