

CHARACTERISATION OF BACTERIAL ENZYMATIC COMPLEX USED IN LEATHER WASTES DEGRADATION

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Abstract

The leather waste generated by the leather industry contains a large amount of hard to degrade proteins. Those squanders are destroyed by incineration and it represents a threat for the environment. Through biotechnological methods, this waste can be used as substrate for enzyme production, the leather serving as the unique source of carbon and nitrogen.

This paper presents the isolation and characterization of leather degrading bacterium. The hydrolytic bacteria where isolated from compost of fur and skin .The isolated colonies show their ability to synthetize various hydrolytic enzyme as proteinase (0.709 – 0.868 U/ml), lipases (20-80 U/ml), collagenases (0.344 – 0.373 U/ml), amylases (0.104 – 0.198 U/ml), keratinases (0.4-1.8U/ml) and it can be use in different biotechnological processes. The proteolytic enzymes have an important value in the biotechnological sector and the obtaining process presented in this paper is a nonpolluting alternative to the current disposal system.

The results show that the isolated bacteria have the capacity to produce hydrolytic enzymes able to degrade different types of leathers.

Key words: degradation, leather wastes, enzymatic product.

INTRODUCTION

Proteolytic enzymes are groups of enzymes that break the long chainlike molecules of proteins into shorter fragments (peptides) and eventually into their components, amino acids (Anson, 1938). Proteolytic enzymes are present in bacteria and plants but are most abundant in animals. Enzymes are biocatalysts produced by living cells to bring about specific biochemical reactions generally forming parts of the metabolic processes of the cells. The isolated bacteria have the capacity to produce proteolytic enzymes using skin and fur as unique source of carbon.

MATERIALS AND METHODS

Materials

Minimal media, Luria Bertani, PCA (plate count agar), CMC (carboximetilcelulose) and starch media was used.

Microorganisms

Three isolated strains from skin and fur compostation were used. The bacterial cultures were growing at 35°C and maintained by periodic transfer on agar tubes with the following composition: peptone 1g%, yeast extract 1g%, NaCl 0.5g% and agar 2g%.

Inoculum growth medium and cultivation conditions

40 mL of minimal media (medium (g/l) 1.0g NaCl₂, 0.05g CaCl₂, 0.7g KH₂PO₄, 0.9g MgSO₄, 2.38g K₂HPO₄, 3.0g sucrose, 0.6g skin and fur) was previously sterilized at 120°C, for 20 minutes and incubated with one loop of isolated bacteria. The inoculum was prepared in 100 mL Erlenmeyer flask by incubation at 35°C, for 120 hours on a laboratory shaker (Heidolph – Germany) at 135 rpm.

Biomass separation

At the end of fermentation process the cultivation medium was centrifuged during 20 minutes at 9000 rpm.

Determination of proteolytic activity

Proteolytic activity was spectrophotometric measured 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 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 was 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.

Determination of lipolytic activity

For the lipolytic activity was used 10 mL emulsion substrate, 2 mL CaCO₂, 5mL citrate buffer, 1 mL enzymatic solution. The samples were incubated 60 min at 37°C, 170 rpm. After incubation the reaction was stopped by adding 20 mL acetone: ethanol 1:1 (v/v).

Lipase activity was measured by titrimetric assay with NaOH 0,1N (Anson, 1938). One unit of lipase activity was defined as the amount of enzyme that release 1 μ mol equivalent of carboxylic groups of fatty acid under analysis conditions (temperature 37°C, pH 7, reaction time 60 min.) (Lupescu et al. 2007).

Determination of keratinolitic activity

Enzyme activity was determined with keratin azure as substrate. The reaction mixture contained 2 mL suspension (1.5 mL Tris Buffer and 0.5 mL culture supernatant) and 80 mg keratin azure (Sigma).

The mix was incubated at 50°C for 60 min and then centrifuged at 10.000 rpm, 20 min. Supernatants were measured at 595 nm. One unit of keratinase activity was define as that amount of keratinase producing an increase of 0.1 absorbent units 595 nm/h (Korkmaz et al., 2004).

Determination of collagenase activity

Collagenase activity was measured by using ninhidrin as substrate following the method of

Moore and Stain (1948). The reaction mix contained 2 mL ninhidrin, monoetil-glicol and eter. After boiling for 30 minutes the samples are diluted with 10 mL n-propanol 50% concentration.

The absorbance was determined at 600nm after 15 minutes.

Determination of amylase activity

The amylase activity was determined. An assay mixture containing 0.5 mL phosphate buffer 0,2M (pH 6.9), starch 1g% and 0.5 mL supernatant. The mixture was incubated 10 min at 30°C. The reaction was stopped using 2 mL DNS. The samples were incubated 5 min at 100°C. After cooling for 12mL distilled water was added. The amylase activity was measured at 546 nm. One unit of amylase activity was defined as the number of μ moles of maltose liberate by 1 mL enzyme solution per minute (Tapai M., 2009).

Determination of organic carbon

For the organic carbon determination 0.5g of skin and fur were introduced in filtered enzymatic solution. It was incubated 48 h at 35°C, 135 rpm. After the incubation it was washed and dried.

The reaction mixture used 10mL K₂Cr₂O₇ and 20 mL H₂SO₄. The samples were incubated for 20 min at 95°C. 150 mL distilled water was added and ortofenantroline was used as reaction indicator. The titration was made with Mohr salt.

RESULTS AND DISCUSSION

Three bacteria strains producing variable proteolytic zone on PCA agar plates where isolated from decomposition of fur and skins: DA7, DA10 and DA13. The clear zone of hydrolysis around each bacterial colony reflects their extent of extracellular proteolytic activity (Habib et al., 2012).



Figure 1. The isolated strains tested on fur and skin into minimal media, after 48 h of incubation

The bacteria were incubated 120 hours in suspension on minimal media, at 35°C, 135 rpm, pH 7.2.

The parameter was performed in fermentation experiments carried out in 100 mL flasks using 40 mL of minimal media.

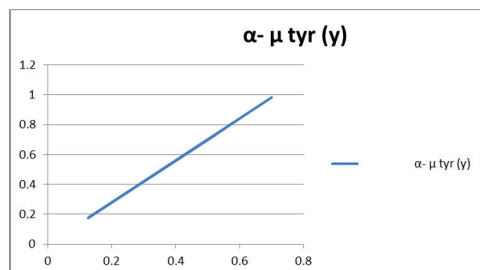


Figure 2. Growth behavior of isolated bacterial strain and rate of extracellular protease production during 120h, after 1:25 dilution

An increase in the cell mass and lipase activity was observed (Figure 3) as a result of a strong aeration (lipase activity = 80 U/mL), pointed out by the lipolytic activity in the above mentioned cultivation conditions.

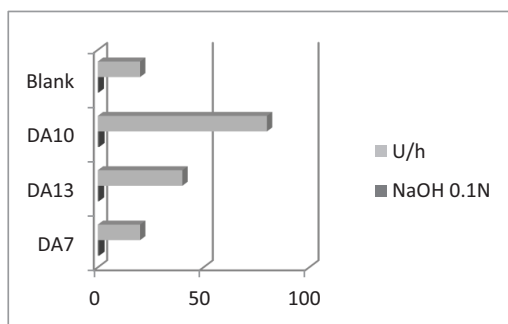


Figure 3. Lipase activity in fermentation media (U/h)

The highest keratinolytic enzyme production by isolated strains was 0.223 U/mL, produced by DA10 strain, in the above mentioned cultivation conditions (Figure 4).

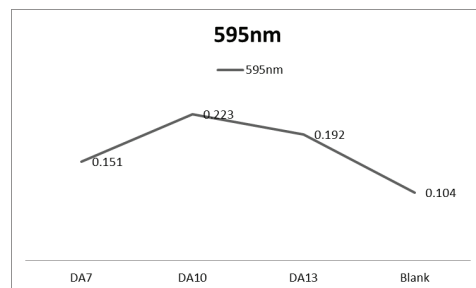


Figure 4. The keratinolytic activity determination at 595 nm

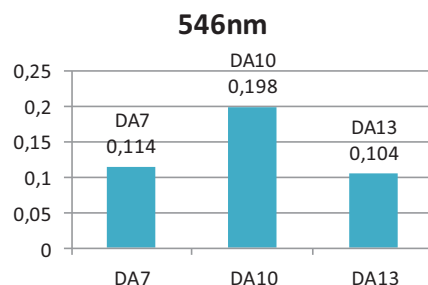


Figure 5. Amylolytic activity of isolates after 120 h of incubation

In submerged fermentation the amylase production has reached maximum of 0.198 U/ml by DA10 strain, at 120 hours of incubation, where it showed a significant increase in enzyme production rate (Figure 5).

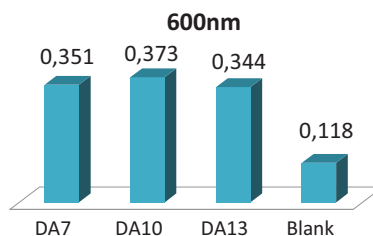


Figure 6. Determination of collagenase activity at 600 nm, after 120 hours of incubation on MM

The collagenazic activity maximum production was 0.373 U/mL, obtained with the DA10 strain in the fermentation medium with

0.6 g of skin and fur as unique source of carbon (Figure 6).

Table 1. Organic carbon determined, after 24 h of incubation of fur and skins in enzymatic solution.

Nr. Crt.	Sample	%
1	Blank	51.68
2	DA7	24.63
3	DA10	24.82
4	DA13	25.75

The organic carbon source has the potential to introduce synthesis of proteases at an optimal level, which was determined through testing of organic compounds, those being utilized as individual resources.

CONCLUSIONS

The isolated strains produced extracellular enzymes which have the capacity to use fur as unique source of carbon. The best results were obtained using DA10 strain.

The strains have high proteolytic activity and are very affective in skins and fur degradation, suggesting its potential use in biotechnological processing involving protein hydrolysis.

BIBLIOGRAPHY

- Anson, M.L., 1938, J. Gen. Physiol. 22, 79-89
- Habib S.M.A., Fakhruddin A.N.M., Begum S., Ahmed M.M., 2012, Isolation and screening of thermostable extracellular alkaline protease producing bacteria from tannery effluents. J. Sci. Res. 4: 515-522
- Korkmaz H., Hür H., Dinçer S., 2004, Characterization of alkaline keratinase of *B. licheniformis* strain HK1 from poultry waste, Annals of Microbiology 54 (2), 201-211.
- Lupescu I., Groposila-Constantinescu D., Jurcoane S., Diguta C., Cozea A., Tcacenco L., 2007, Production of lipases by strain of the non-conventional yeast *Yarrowia lipolytica* and isolation of crude enzyme, Roumanian Biotechnology letter, Vol 12, No3, pp 3261-3268.
- Moore S., Stein W.H., 1948, Photometric ninhydrin method for use in the chromatography of amino acids, J. Biol. Chem. 176: 367-388.
- Tapai (Stoica) Mihaela, 2009, Biosinteza unor amilaze utilizate în hidroliza unor materii prime agricole, Teza de Doctorat, p.65-66, Bucuresti.