AMYLASES AS BIOLOGICALLY ACTIVE SUBSTANCES PRODUCED BY BACTERIAL STRAINS COLLECTED FROM POLLUTED AREAS

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Abstract. Amylases are among the most important enzymes, with applications in food, pharmaceutical and textile industries. The present study aimed to render the ability of bacterial strains isolated from environments contaminated with heavy metals or petroleum products to synthesize amylases. Three of the strains studied are able to synthesize amylases; two of them belong to the genus *Pseudomonas* and one was classified in the genus *Bacillus*. Enzymatic activity of these three bacterial strains (*Pseudomonas* sp. HM2, *Pseudomonas* sp. OP16 and *Bacillus* sp. OP3) is favourable and *Bacillus* strain sp. OP3 may represent the object of new applicative studies.

Keywords: biologically active substances, bacteria, amylases.

Rezumat. Amilazele ca substanţe biologice active produse de tulpini bacteriene prelevate din zone poluate. Amilazele sunt printre cele mai importante enzime, cu aplicabilitate în industriile alimentară, farmaceutică şi textilă. Prin prezentul studiu s-a urmărit capacitatea unor tulpini bacteriene izolate din medii contaminate cu metale grele sau cu produşi petrolieri de a sintetiza amilaze. Trei dintre tulpinile luate în studiu au capacitatea de a sintetiza amilaze, două dintre acestea aparţinând genului *Pseudomonas,* iar una fiind afiliată genului *Bacillus.* Activitatea enzimatică, la cele trei tulpini bacteriene (*Pseudomonas* sp. HM2, *Pseudomonas* sp. OP16 şi *Bacillus* sp. OP3) este favorabilă, tulpina de *Baciluss* sp. OP3 pretându-se la noi studii cu potenţial aplicativ.

Cuvinte cheie: substanţe biologic active, bacterii, amilaze.

INTRODUCTION

Amylases are among the most important enzymes (NAIDU & SARANRAJ, 2013); those amylolytic enzymes are of great significance in food, textile, paper, fermentation, pharmaceutical and sugar industries (KUNAMNENI et al., 2005; WINDISH & MHATRE, 2012).

Amylases can be found in plants, animals, and microorganisms (BEN MASSOUD et al., 1999; OSFAR & TRI, 2011). Amylases can be obtained from: yeast, bacteria, actinomycetes and several fungi (DAMODARA et al., 2012). Among bacterial sources, we can mention: *Bacillus* genus with *B. subtilis, B. stearothermophilus, B. licheniformis* and *B. amyloliquefaciens,* and some bacteria of the genus *Rhodothermus, Lactobacillus* (MONTEIRO DE SOUZA & MAGALHÃES PÉROLA, 2010), *Pseudomonas stutzeri* (MAALEJ et al., 2014).

MATERIAL AND METHODS

The biological material consisted in 25 bacterial strains isolated from areas contaminated with petroleum products and heavy metals.

The selection of the bacterial strains, which have the potential to synthesize amylases, was made on average with starch nutrient agar medium, for 48 hours at 28 °C. After incubation, the plates were flooded with Lugol solution; if starch hydrolysis occurs, a yellow hollow appears around the colonies (LAZĂR et al., 2004).

The bacterial strains were submitted to the protocol for determining the Gram appurtenance, using Gram microbiology technique (LAZĂR et al., 2004); taxonomic identification was made using Biologic GN2 and GP2 micro plates. Micro plates were inoculated at 28 °C for 24 hours, according to the manufacturer's recommendations (Biologic, Inc).

Enzymatic activity was determined after the method described by LAZĂR et al. (2004), using the spectrophotometric method, reading the samples values at 580 nm.

Bacterial cell was cultivated on liquid nutrient agar medium and incubated at 28 °C on a rotary shaker (150 rpm), for five days.

Amylases activity by Zymography was made by gel on a thin 2 % agarose and 1 % soluble starch in Millis-Q water. In the gel were carried holes, which was added 8 µl supernatant. The gel was incubated at 60 °C for 60 minutes and then flooded with Lugol.

RESULTS AND DISCUTIONS

All bacterial strains were cultivated by starch nutrient agar medium, at 28 °C for 48 hours. After flooding plates with Lugol solution, only 5 bacteria strains gave positive reaction (Fig. 1), which means that 40 % of the strains have the ability to synthesize amylases.

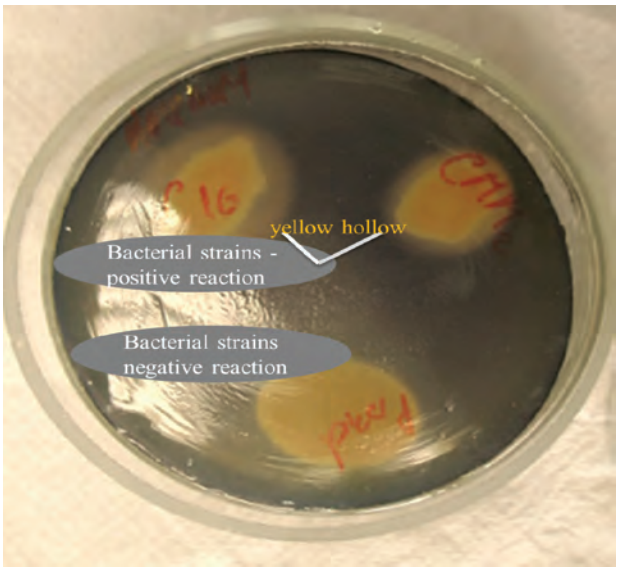

Figure 1. Amylases - synthesis by the bacterial strains.

The five amylolytic bacteria have been identified by using Biologic Systems. Because two bacterial strains were Gram-negative and three Gram-positive, it was used Biologic CN2 micro plates for Gram negative bacteria and CP2 micro plates for Gram positive bacteria. The results based on this biochemical tests indicated that two strains belong to the genus *Pseudomonas* and three were classified in the genus *Bacillus*. It is known that amylases are mainly derived from the genus *Bacillus* (MONTEIRO DE SOUZA & MAGALHÃES PÉROLA, 2010).

After having established the affiliation of the amylolytic bacterial strains, we can note: *Pseudomonas* sp. HM2, *Pseudomonas* sp. OP 16, *Bacillus* sp. OP 3, *Bacillus* sp. OP 9, *Bacillus* sp. OP 6 (indicative: HM - heavy metals, OP - oil pollution and experimental number).

Following the evaluation of enzymatic activity (Table 1), enzymatic activities was identified in three of the five bacterial strains studied at the first screening. All five strains have amylolytic activity.

Table 1. Enzyme activity of amylolytic bacterial strains.

UE (enzyme activity unit)					
Bacterial strains	24 h	48 h	72 h	96 h	120 h
Pseudomonas HM2	2.514	5.88	4.22	10.02	10.88
Pseudomonas OP16	1.961	6.55	7.352	14.35	11.6
Ps. aeruginosa	0.05	0.21	0.01	0.001	0.09
Bacillus OP 3	2.53	9.099	21.71	24.03	22.01
Bacillus OP 9	0.412	0.3814	0.21	0.21	0.02
Bacillus OP 6	1.141	0.511	0.41	0.43	0.39

The bacterial strains *Pseudomonas* sp. HM2, *Pseudomonas* sp. OP16 have a low enzyme activity compared with the strain *Bacillus* sp. OP3 which has an enzymatic activity similar with other strains of the genus *Bacillus* (OSFAR & TRI, 2011).

Bacillus sp. OP9 and *Bacillus* sp. OP6 do not show enzymatic activity similar to *Ps. aeruginosa*, a negative control; this bacterial strain cannot metabolize starch.

These results prompted us to test the enzymatic activity of the supernatant by zymography method (Fig. 2).

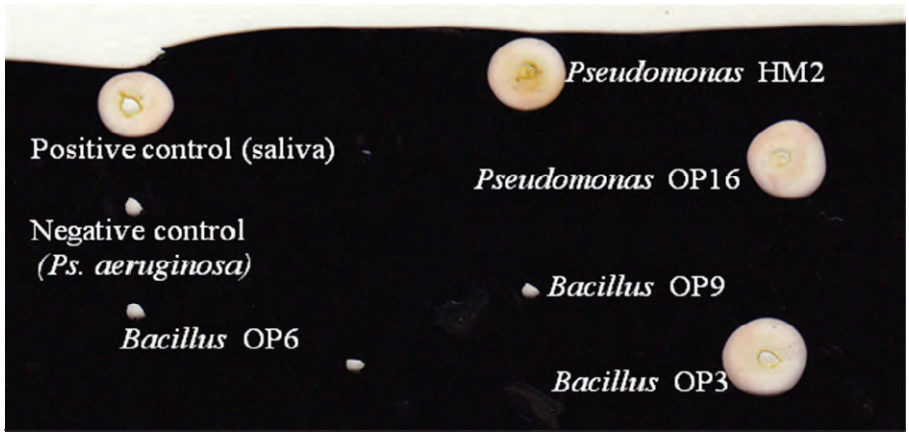

Figure 2. Amylase activity by Zymography.

The results of amylase activity by zymography of the bacterial strains confirm previous data; *Bacillus* sp. OP 9 and *Bacillus* sp. OP 6 strains do not have enzymatic activity.

CONCLUSIONS

There have been isolated from contaminated environments, three bacterial strains: *Pseudomonas* sp. HM2, *Pseudomonas* sp. OP16 that are dependent on enzymes for metabolising carbohydrates of the starch type. Bacterial strains, *Bacillus* sp. OP3 show a higher enzyme activity compared with the other two bacterial strains (*Pseudomonas* sp. HM2, *Pseudomonas* sp. OP16).

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