EXO-β-1,4 – GLUCANASE ACTIVITY IN SOME BACTERIAL STRAINS

SIMONA DUNCA[1*], ERICA NIMIȚAN[1], OCTĂVIȚA AILIESEI[1], MARIUS ŞTEFAN[1], ZENOVIA OLTEANU[2]

Keywords: cellulolytic bacteria, cellulose, exo-β-1,4-glucanase

Abstract: The capacity to synthesize cellulolytic enzymes is widely met in microorganisms; however a very limited number is capable of producing high quantities of cellulases. A number of 22 cellulolytic bacterial strains were isolated from different natural media (forest soil, straw, sawdust and manure). By quantitative screening, three highly-productive exo-β-1,4-glucanase strains were isolated.

INTRODUCTION

The increasing importance paid nowadays to cellulases is due to their use in zootechny, agriculture and various fermentation industries using cellulose waste as raw material. Because of the major role played by the enzymes of cellulolytic complex in different industrial fields, the current research studies are oriented towards the isolation and selection of some active microorganisms from natural media and the improvement of biosynthesis results by optimization of the growth conditions or by mutagenesis.

The natural degradation of the cellulose substances is carried out by microorganisms using different strategies, according to their characteristics. The capacity to synthesize cellulolytic enzymes is widely met in microorganisms; however a very limited number is capable of producing high quantities of cellulases (Luiza Jescu, 1995), thus contributing to a non-polluting recycling of cellulose waste (G. Mencinicopschi, 1980).

Numerous data on microorganisms causing the hydrolysis of cellulose show that a multiple enzyme system is involved in this process, with various modes of action (endo- and exo) and different dimensions which reflect the heterogeneity of cellulose structure and organization. Literature data specify that cellulases are produced by various microorganisms, particularly by bacteria and filamentous fungi. Despite the fact that the cellulosolytic bacteria (belonging to the genera *Clostridium*, *Cellulomonas*, *Bacillus*, *Bacteroides*, *Thermomonospora*, *Erwinia* and *Acetivibrio*) synthesize smaller amounts of enzymes than the fungi, they are more and more used both because of the simple growth conditions, and of the genetic manipulation facilities (Luiza Jescu, 1995).

AIM OF INVESTIGATIONS

This paper contains data on the isolation of cellulolytic bacteria from various natural media and the study on the exo-β-1,4-glucanase activity with a view to selecting the highly cellulase-productive strains.

MATERIALS AND METHODS

In order to isolate cellulase-producing bacterial strains, samples were collected from various natural media: forest soil, straw, sawdust and manure. Sampling was performed in aseptic conditions in July and September 2003. Using the collected samples, successive dilution-suspensions in sterile water were prepared, with a dilution coefficient of 10. Using the last five dilutions (10^{-5} – 10^{-10}) inoculations of the media distributed in Petri dishes (three dishes per each dilution) were carried out, by spreading the inoculum on the surface. Two types of culture media were used, differentiated by the carbon source (enzyme inductor) as follows: gelose with cellulose powder 1% and gelose with carboxymehtylcellulose 1%. The inoculated dishes were incubated at 28° C for 5-7 days. After 7 days, the bacterial colonies started to appear on the surface of culture medium; the colonies with cellulosolytic activity (the ones exhibiting a clear, transparent area around due to cellulose enzymatic hydrolysis) were recorded. The isolated strains were purified and conventionally marked with the letters S (for those isolated from soil), P (for those isolated from straw), R (sawdust) and B (manure).

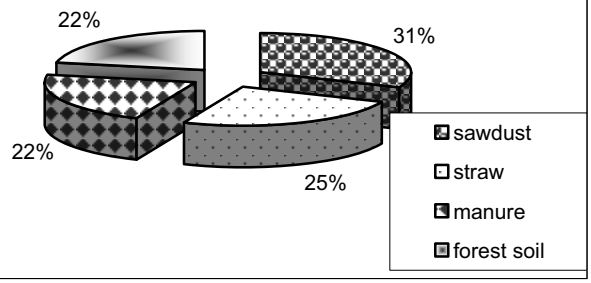
The microscopic examination of coloured smears using Gram method consisted in establishing the morphology of the isolated strain.

With a view to selecting the best cellulase-producing strains, exo-β-1,4-glucanase activity was determined in the isolated strains. For the determination, the bacteria under examination were grown in submerged cultures under stationary conditions, at 28° C for 48 hours, using a liquid medium containing 1% of cellulose powder. Of the enzymes of the cellulolytic complex, exo-glucanase (1,4-β-cellobiohydrolase) E.C.3.2.1.9.1. was determined, an enzyme which catalyzes the hydrolysis of the 1,4-β-D-glucosidic links from cellulose, releasing cellobiose from the non-reducing ends of the chain. The method of determination involves mainly the dosing of the reducing sugars resulted from the hydrolytic action of the enzymatic preparation on Whatman paper no. 1 (Pettersson method). The results are expressed as U/ml/h (mg of glucose released by the action of the enzyme on the substrate for one hour at 50° C). The specific activity was also calculated by referring the enzymatic activity to the quantity of protein determined by the Lowry method.

RESULTS AND DISCUSSIONS

A number of 22 bacterial strains were isolated in pure cultures from the examined samples (forest soil, straw, sawdust and manures) (Photo 1-4). Most strains were isolated from sawdust (10), 8 from straw, and 7 from manure and garden soil (Fig.1).

Fig. 1 - Cellulolytic bacterial strains isolated from different natural media



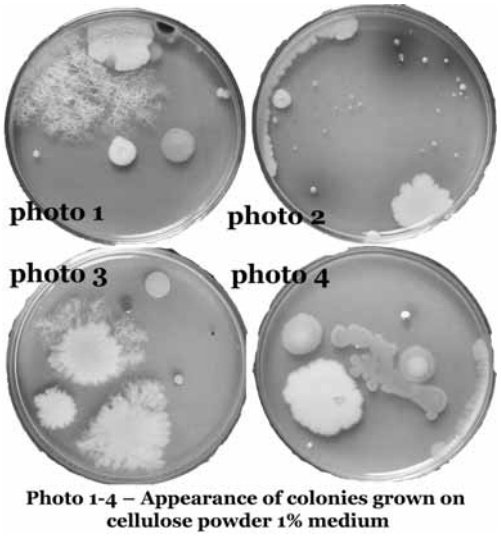


Photo 1-4 – Appearance of colonies grown on cellulose powder 1% medium

In what concerns the growth of the strains on the two types of medium (gelose with 1 % cellulose powder, and, respectively with 1 % carboxymehtylcellulose), a much better growth was noted on the cellulose powder medium, which proves that insoluble cellulose is the best carbon source, acting as an inductor in cellulase synthesis.

The microscopic examination revealed that most of the strains examined are morphologically Gram-positive sporulated bacilli (the spores have different sizes, sub-terminal, terminal or central position, and are isolated, grouped in short or long chains) – Photo 5-8. Gram-negative bacilli and actinomycetes were also identified.

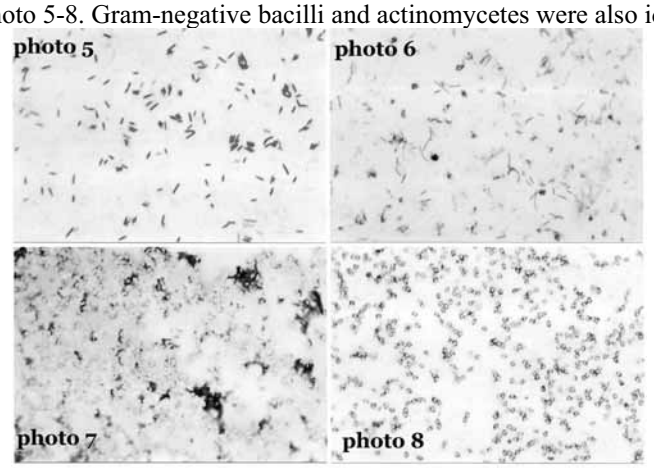


Photo 5-8 – Micro-morphological appearance of some of cellulolytic strains isolated

The results obtained for the exo-β-1,4-glucanase activity are indicated in the Table I and Fig. 2-5.

Table I – Exo-β-1,4-glucanase activity (U/ml/h) in the isolated strains

Strain	exo-β-1,4-glucanase activity (U/ml/h)	Total protein(mg protein/ml)	Specific activity (U/mg protein x 10^{-3})
P_1	0,0240	2,852	8,415
P_3	0	2,744	0
P_4	0,0222	2,765	8,028
P_5	0	2,687	0
P_7	0,0226	2,777	8,138
P_8	0,0409	2,833	14,437
S_2	0	2,576	0
S_3	0,0047	2,682	1,752
S_4	0,0233	2,722	8,560
S_5	0,0029	2,886	1,012
S_7	0,0262	2,965	8,836
B_1	0,1087	2,792	37,704
B_5	0,0136	2,883	4,717
B_6	0,0057	2,851	1,999

Strain	exo-β-1,4-glucanase activity (U/ml/h)	Total protein(mg protein/ml)	Specific activity (U/mg protein x 10^{-3})
R_1	0,0258	2,865	9,005
R_2	0,0032	2,799	1,143
R_3	0	2,906	0
R_3'	0,0086	2,635	3,263
R_4	0	2,674	0
R_5	0	2,637	0
R_7	0,0079	2,745	2,878
R_8	0,0352	2,494	14,114

Fig. 2 - Exo-β-1,4-glucanase activity (%) of the cellulolytic strains isolated from straw

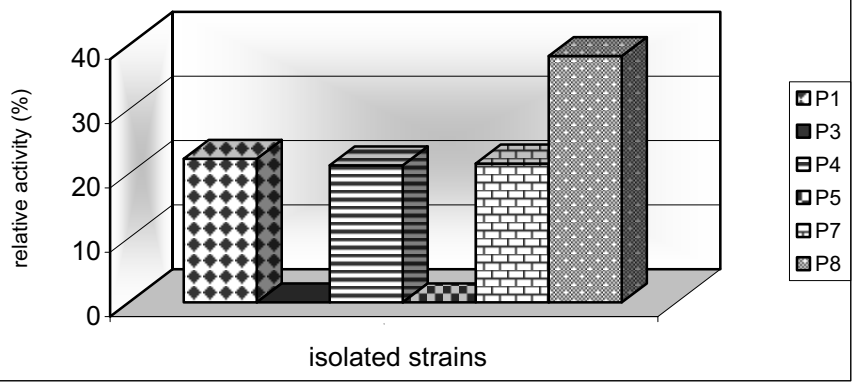


Fig. 3 - Exo-β-1,4-glucanase activity (%) of the cellulolytic strains isolated from forest soil

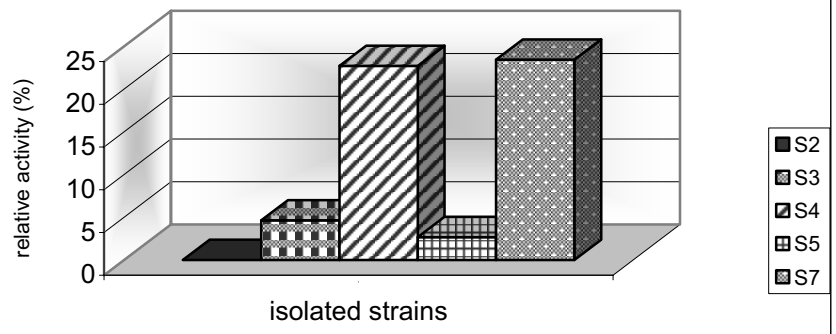


Fig. 4 - Exo-β-1,4-glucanase activity (%) of the cellulolytic strains isolated from manure

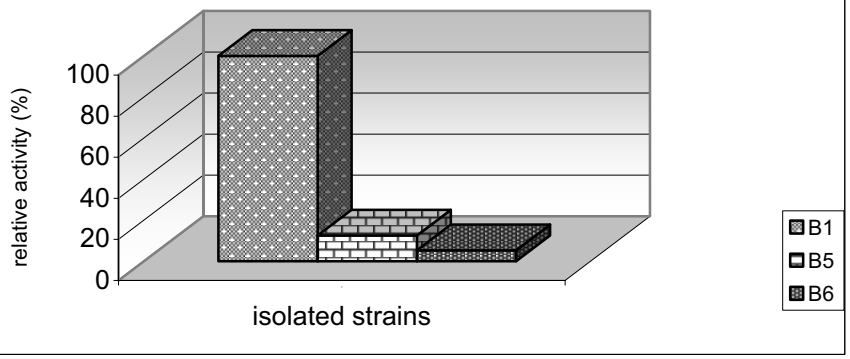
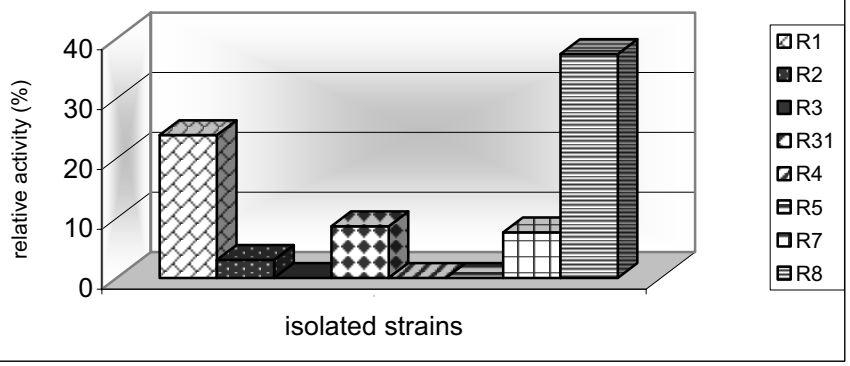


Fig. 5 - Exo-β-1,4-glucanase activity (%) of the cellulolytic strains isolated from sawdust



The data exhibit significant differences in the activity of the 22 strains subjected to examination. Except a number of six strains in which this enzymatic activity was not identified all other strains synthesized the enzyme differently, depending on the strain. Thus, B_1 was remarked by the highest exo-β-1,4-glucanase activity (37.704 U/mg protein x 10-3). Strains P_8 and R_8 are also quite highly exo-β-1,4-glucanase-producing, with activities of 14.437 and 14.114 U/mg protein x 10^{-3} respectively. The rest of the strains were arbitrarily classified by us as low-producing, their activities ranging between 1.012 – 9.005 U/mg protein x 10^{-3}.

Our future research studies aim at testing the other components of the cellulosolytic enzymatic system (endo-β-1,4-glucanase and β-glucosidase) in the three selected strains, their taxonomic classification, isolation of new microorganisms exhibiting high cellulosolytic activity as well as the testing of some new cellulose material sources.

CONCLUSIONS

A number of 22 cellulolytic bacterial strains were isolated from different natural media (forest soil, straw, sawdust and manure).

By quantitative screening, three highly-productive exo-β-1,4-glucanase strains were isolated.

REFERENCES

Artenie, V., Elvira Tănase, 1981 – Practicum de biochimie generală, Ed. Univ. „Al. I. Cuza" Iaşi, pp. 228-230

Beguin, P., Aubert, J.P., 1994 – The biological degradation of cellulose - FEMS Microbiol. Rev., 13, 1: 25

Luiza Jescu, 1995 – Celulaze de origine microbiană, St. Cerc. Biochim., 38, 1-2, pp. 65-78

Mencinicopschi, G., Milena Stoica, Viorica Ionescu, 1979 – Cercetări preliminare privind izolarea şi testarea unor microorganisme producătoare de celulaze, Lucrările celui de-al II-lea Simpozion de Microbiologie Industrială, Iaşi, 25-26 mai, pp. 246-250

Moreira, A.R., Philips, J.A., Humphrey, A.E., 1981 – Utilization of carbohydrates by Thermomonospora sp. grown on glucose, cellobiose and cellulose, Biotechnology and Bioengineering, XXIII, 6, pp. 147-150

Lidia Popa, Elena Enache, 1977 – Studiul activității celulazice a unor mucegaiuri pe medii cu diferite surse de celuloză, Lucrările primului simpozion de microbiologie industrială 1976, pp. 176-181

[1] "Al.I.Cuza" University of Iaşi", B-dul Carol I, 11A 6600, Iaşi-România
[2] Institute of biological research, Iasi
[*] corresponding author: sdunca@uaic.ro