CELLULOLYTIC MICROORGANISMS ISOLATION FROM DIFFERENT NATURAL HABITATS

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Abstract: Cellulose represents the main polymeric component of plant matter and is also the most abundent polysaccharide on Earth. In cellulose-degrading ecosystems, a variety of celluloytic fungi and bacteria exist, which act synergically, in order to convert insoluble, cellulose-based substrate to soluble sugars – cellobiose and glucose mainly – which afterwards are assimilated by the cells. Thus, we investigated the presence of such microorganisms in various natural habitats.

INTRODUCTION

The development of efficient methods for biomass degradation, and also for conversion of sugars to valuable products such as butanol, aminoacids and usable energy forms such as ethanol and methane could lead to a much lowered degree of petrol-based fuels and chemicals dependency. (Doi, 2003).

The production of fuels from cellulosic biomass, an abundent and renewable resource, might offer huge benefits in terms of durability, security and economic growth. Waste from agricultural, wood, food and paper processing industry may be utilised for the purpose of obtaining ethyl alcohol, single-cell proteins and/or acetone and organic acids. (Georgescu, 2004).

In this paper, we have investigated the presence of microoganisms which degrade cellulose within 6 types of natural habitats from the perimeter of the Botanical Garden of Iasi.

MATERIAL AND METHODS

In order to isolate bacterial strains with cellulolytic activity, we selected vegetable residues found in different decomposition stages. The samples were taken from the perimeter of the “Anastasie Fătu” Botanical Garden of Iasi, from 6 different isolation sources:

No.	Isolation source
1.	Fresh hay
2.	Degraded hay
3.	Manure
4.	Decomposing leaves
5.	Manure mixed with straws
6.	Compost

Decimal seriated suspensios-dilutions were prepared from the taken samples. For qualitative screening, we used a cellulose-Congo Red type medium (Hendricks, Doyle and Hugley, 1995), containing:: K_2HPO_4 0,5 g; $MgSO_4$ 0,25 g; microcrystalline cellulose 1,88 g; Congo Red 0,20 g; Noble agar 5,00 g; gelatine (Difco) 2,00 g; soil extract 100 ml; distilled water 900 ml; pH = 7,0. Medium autoclaved at 1 atm., 30 min.

The advantage of using this type of medium is that the Congo Red facilitates lysis area observation, as the colorant forms a complex with the nonhydrolised polysaccharides. (Teather and Wood, cit. Hendricks, 1995).

Inoculation was performed using three of the seriated dilutions (10^{-3}, 10^{-4}, 10^{-5}), onto the surface of the medium, in Petri dishes. For each dilution, two dishes were inoculated, the medium in one dish containing cellulose, the other, cellobiose. This led to a total of 36 dishes for the 6 isolation sources.

After 48 hours from incubation, the colonies exerting celluloytic activity have been surrounded by clear halos, representing areas of cellulose hydrolyisis, having various diameters, depending on the enzymatic activity of each tested strain. Later, the hydrolysis areas for both cellulose and cellobiose were measured, and the colonies with higher levels of activity have been transfered onto fresh medium. Eventually, the cellulolytic activity was tested by measuring the activity of glucanohydrolase, cellobiohydrolase and β-glucosidase.

RESULTS AND DISCUSSIONS

Cellulase production potential was first tested by growth in Petri dishes, on medium containing 1.88% cellulose (as sole source of carbon and enzyme inductor), for 48 hours at 28°C. Cellulolysis has been indicated by observing clear areas on the surface of the culture medium. The medium, containing Congo Red, has an uniform, reddish colour, but due to

cellulolytic activity, clear halos appear around bacterial colonies. These areas show the hydrolysis of cellulosic substrate through the activity of produced cellulase. (Photos 1, 2, 3, 4).

Photo 1. Cellulose hydrolysis area for strain 11CB

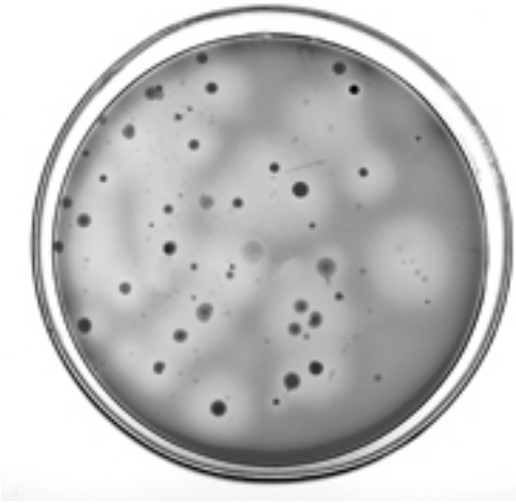

Photo 2. Cellulose hydrolysis area for strain 31CB

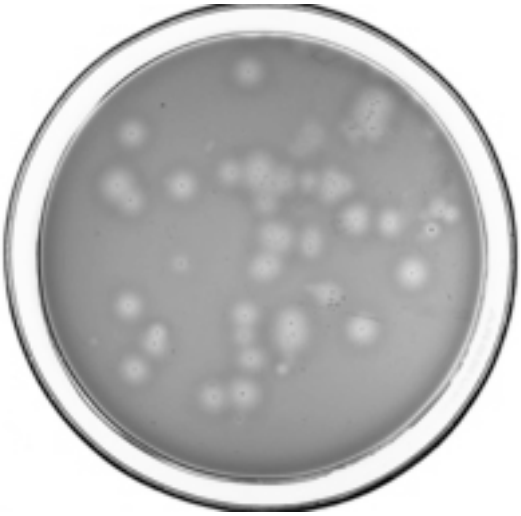

Photo 3. Cellulose hydrolysis area for strain 22CB

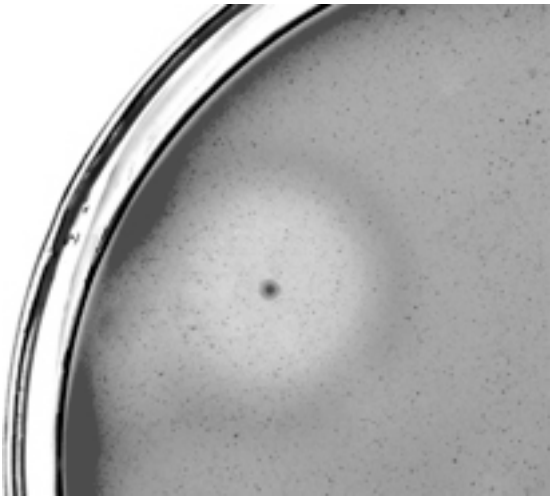

Photo 4. Cellulose hydrolysis area for strain - detail

From the samples taken (I, II, III, IV, V, VI), 16 actively producing cellulase strains were isolated in pure cultures, which presented different diameters of the hydrolysis area.. The diameters were measured at 24, 48 and 72 h from inoculation, and we found that the conclusive values were the ones for 48 and 72 h respectively. According to the observations, the 16 strains could be grouped by the diameter of the cellulosyic substrate hydrolysis area: 7 strains with a diameter between 5 and 10 mm; 5 strains with a diameter between 10.1 and 15 mm; 3 strains with a diameter between 15.1 and 20 mm; 1 strain with a diameter between 20.1 and 25 mm.

By computing the diameter of the hydrolysis area to the diameter of the corresponding colony ratio (**R= Dhz/Dc**), the colonies were grouped as follows: high potential cellulase producer (R > 3,5) – 2 strains (12,5 %);

moderate potential producer (R = 2 – 3,5) – 8 strains (50 %); poor potential producer (R <= 2) – 6 strains (37,5 %). (Fig. 1)

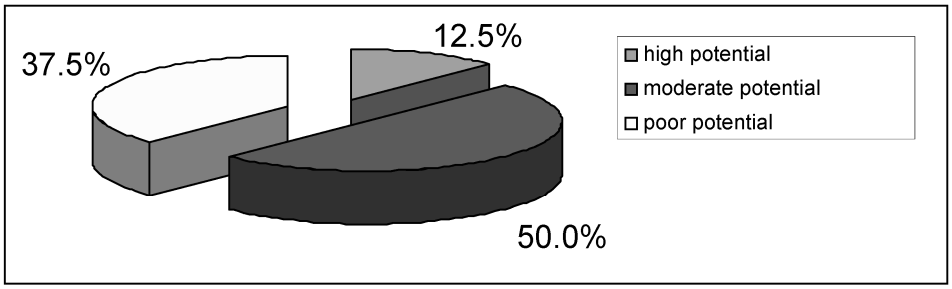

Fig. 1. Percentile representation of isolated bacterial strains, by the cellulase producing potential

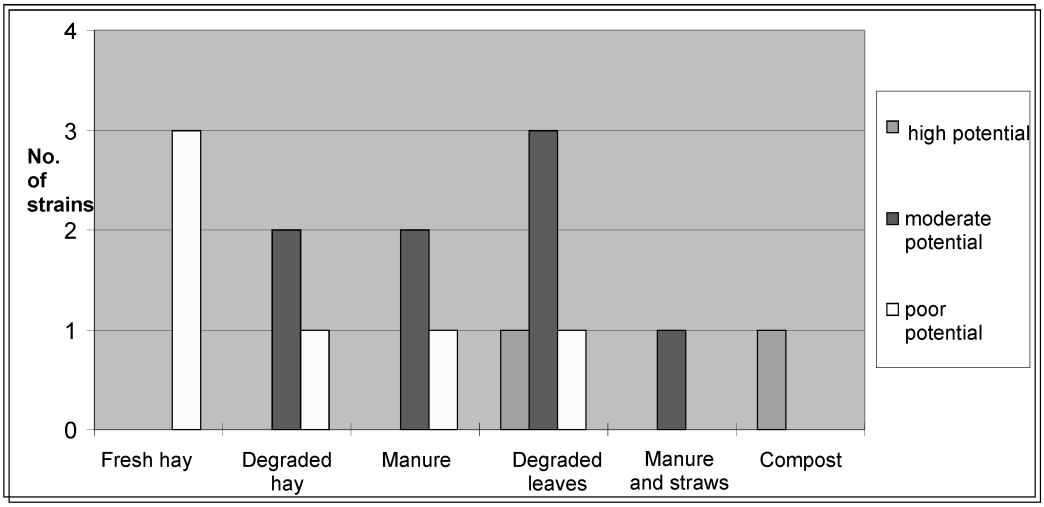

Fig. 2. Isolated bacterial strains representation by source of isolation and cellulasic complex enzymes biosinthetic potential

Pertaining the source of isolation, a high enzymatic activity (increased cellulase production) was identified for the strains which were isolated from two specific types of substrate, the graph of enzymatic activity repartition per isolation sources being shown in Fig. 2.

Regarding the carbon source which was used, 11 out of 16 strains grown on medium containing cellulose and 5 strains grown on medium containing cellobiose were selected.

By correlating the nature of the carbon source with the computed diameters, we can see that the strains grown initially on medium with cellobiose belong to the poor potential and moderate potential groups, while amongst the strains initially grown on medium with cellulose there are high cellulase production potential strains. (Fig. 3).

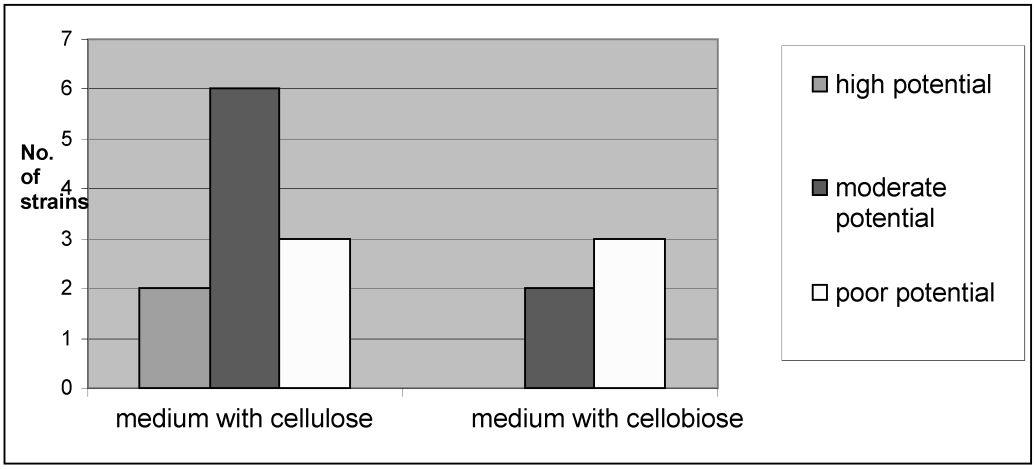

Fig. 3. Representation of isolated bacterial strains according to the carbon source used in culture medium and the cellulasic complex enzyme synthesis potential

From the macroscopic study of isolated colonies we observed that their type and aspect are mainly similar, and generally they were rounded aspect, smooth and shiny surface, regulated edges (S type) colonies, with a brown or red colour, varying by intensity. All colonies exerted weak substrate adherence. Microscopic study shows the presence of both coccoid and bacillar forms, 69% cocci and 31% bacilli (Photos 5, 6, 7, 8, 9, 10)

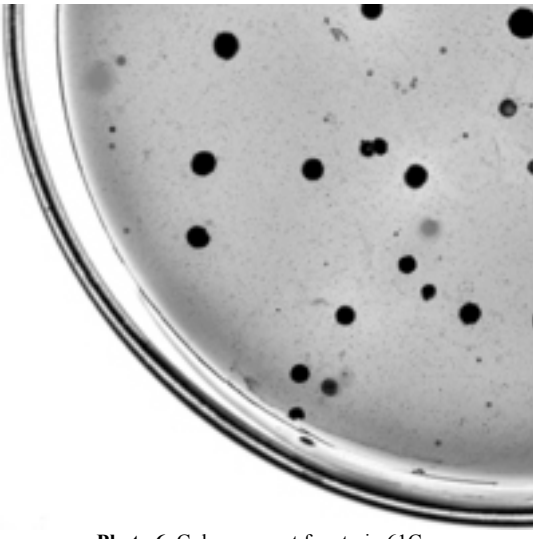

Photo 6. Colony aspect for strain 61C

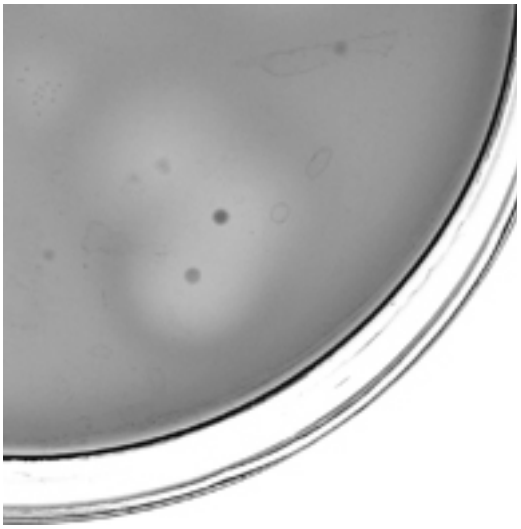

Photo 5. Colony aspect for strain 12CB

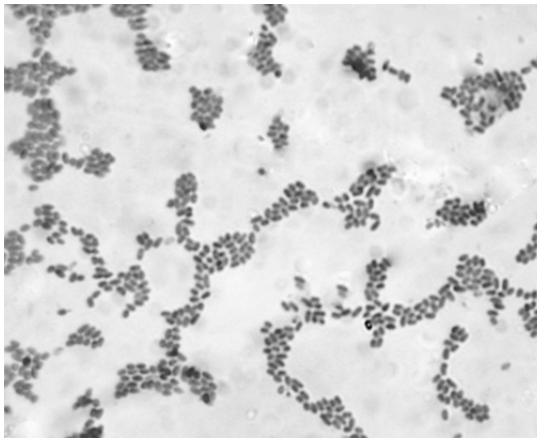

Photo 7. Strain 12CB – microscopic view (x 1000)

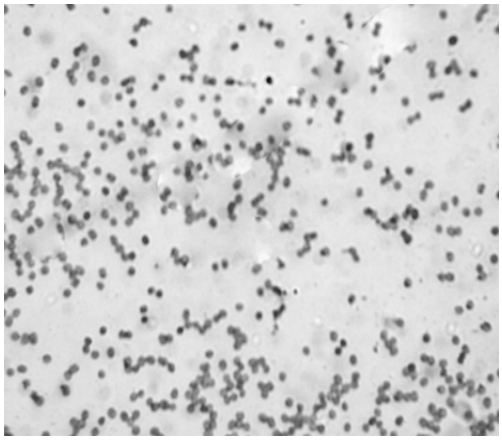

Photo 8. Strain 23C – microscopic view (x 1000)

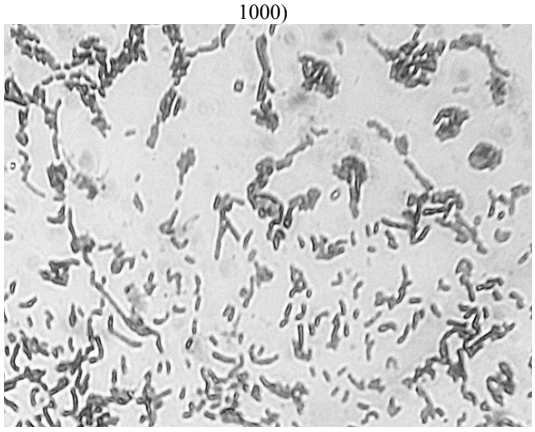

Photo 9. Strain 42C – microscopic view (x 1000)

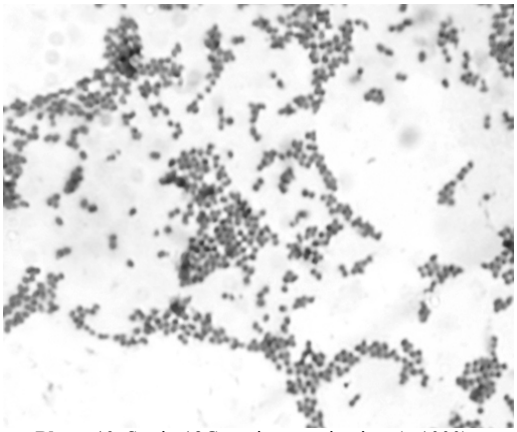

Photo 10. Strain 13C – microscopic view (x 1000)

Out of 16 strains which have shown cellulolyitc activity, 4 of them were subjected to testing for enzymes dosage, as they had the best cellulase production potential: strains 11C, 21C, 32CB, 44C. Testing was done using Weimer & Zeikus medium (1977). After investigating the enzymatic complex represented by glucanohydrolase, cellobiohydrolase and β-glucosidase, a certain potential of celullase synthesis was indicated for strains: 32CB, initially grown on medium with cellobiose (0,0657 U/ml/min.) and 44C, initially grown on medium with cellulose (0,0529 U/ml/min.).

CONCLUSIONS

Following our investigations, we determined that the best sources of isolation for bacterial strains with high cellulase producing potential is represented by decomposing leaves and compost. The cellulolytic activity of bacterial strains has been influenced by the carbon source used in culture medium, strains grown on medium with cellulose showing, in general, bigger hydrolysis areas compared with the strains grown on medium with cellobiose. After determining cellulolytic activity by biochemical means, two strains with higher enzymatic activity were identified: strain 32CB and strain 44C.

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