Microbiology

Selection of Industrially Valuable Xylanase Producer Strain

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ABSTRACT. Xylanase (hemicellulase) is one of the key enzymes in the creation industrial process of natural biopolymers enzymatic degradation. The importance of this enzyme from the viewpoint of alternative sugar and biofuel production from plant row materials is attracting more and more attention. The objective of the present study was the primary selection of xylanase producing fungi from soil - rich microflora sources of the Caucasus, selection and optimization of the carbon content and nitrogen components in nutrient media under submerged fermentation for industrial xylanase production. Initially, fungal strains, including extremophiles, were isolated from different soils of climatic zones of Georgia. In total, more than 420 strains were isolated and purified. Among them 85 were defined as extremophilic strains (halophiles). Finally, 21 most active producers of xylanase were chosen from this collection. Further selection revealed five strains representing the following genera: *Aspergillus*, *Penicillium*, *Sporotrichum* and *Trichoderma* according to their relatively high activity of extracellular xylanase (with maximum 52 U/ml, *Penicillium canescence* AEM 85). Nutrient medium for xylanase cultivation in submerged condition for *P. canescene* AEM 85 was optimized. The xylanase activity selected producer, while the growth on the optimized medium as compared with the basic medium, increased by more than 40%. *© 2015 Bull. Georg. Natl. Acad. Sci.*

Key words: *xylanase, microscopic fungi, optimization, submerged fermentation.*

Xylan (hemicellulose) requires synergistic action of endo- and exo- hemicellulases for complete hydrolysis to constituent monomers-penthouses. Xylan is predominantly present in plant cell walls and in combination with cellulose and nonsugar polymer lignin represents more than 80% of plant biomass. Enzymes decomposing plant biopolymers are synthesized by different varieties of microorganisms, mainly eukaryotes. For the industrial application of xylanases, filamentous fungi seems to be the best source due to their high activity and content of the full set of xylan-degrading enzymes (microscopic and basidial fungi) [1].

According to the existing information, xylan is the best inducer for xylanase biosynthesis. The use of purified xylan in large amount to induce xylanase

microbial synthesis significantly increases the cost of enzyme. Therefore, for commercial production of the enzyme, a bioprocess to produce xylanase in high quantities by growing the producers on inexpensive substrates was developed [2].

In nature, mycelial fungi play special role in decomposition process of renewable plant biopolymers due to their potential to form wide spectrum of enzymes degrading plant biopolymers such as cellulose, xylan, starch, lignin, pectin, innulin, etc. One of the advantages of mycelial fungi, as enzyme producers, is their ability to form simultaneously lignin degrading enzymes as active complex of cellulases and xylanases. They create the conditions for maximal degradation of wooden biopolimers to low molecular weight mono- and oligocomponents. Stable enzymes from extremophiles, which determine their prolonged and successful application, are especially attractive for such process.

For the last twenty years selection of microorganisms has obviously shown that search for stable forms of wooden biopolymers degrading enzymes is expedient among the microorganisms inhabiting under comparatively critical-extreme conditions [3].

Annually, large quantities of lignocellulosic wastes - substrates for xylanases, cellulases, ligninase, wooden biopolymers degrading enzymes, billions of tons of which (agricultural and agro-industrial residues, like wheat bran, sugarcane bagasse, soybean, rice straw, corncob, orange peel, etc.) are generated through industrial processes such as: agroprocesses, breweries, paper-pulp, textile and timber industries. Their disposal becomes a problem regarding space, causing environmental pollution because most of the wastes undergo burning. However, the plant biomass regarded as “wastes” is biodegradable and represents an inexpensive alternative source for the production of huge amount of fermentable sugars. Such “wastes” could be effectively used for microbial growth and enzymes synthesis [4, 5], creation of technologies for biofuel, organic solvents, sugars and by microbial transformation of agro “wastes” production of the number of secondary metabolites.

Materials and Methods

Fungi strains were selected from different ecological niches of the Caucasus region and its southern slopes, particularly alpine zone – mountainous region of Racha, altitude 1500 meters (raw humus calcareous); subalpine zone – high mountain Kazbegi region, at the elevation of 2000 meters (mountain-meadow soils); humid subtropical climate zone – the Black Sea beach, Poti region (lowland bog soil and podzols); steppe zone – continental climate Sighnaghi region, with altitude of 500 meters (black and chestnut soils); semi-desert zone – Marneuli region, altitude 600 meters (chestnut, alkalized and nitric soils), dry subtropical zone – Telavi region, with altitude 700 meters (brown and black soil, alluvial soil), continental climatic zone – Borjomi region (brown forest podzols, volcanic soils).

At the initial stage, strains were grown and developed on solid agar beer syrup nutrient medium of the following composition (on 1 l): 0.5 l beer syrup 7 B, 0.5 ordinary water, 20.0 g agar-agar, (pH – 5.5-6.0). Nutrient medium poured in flasks was sterilized during 40 min at 0.7 atm. Flasks were kept into thermostat, in which temperature corresponded to the optimal growth of temperature required for each strain. Simultaneously, optimal temperature and pH needed for growth and development for identified strains were established; the effect of different concentrations of salt (NaCl) on the growth and development of cultures was studied. Temperature and pH optimums were established in relation to maximal growth of fungi strains that was defined by colony diameter and growth speed.

In order to reveal halophilic cultures and determine their galophilicity, different concentrations of NaCl from 0.5 M to 4.0 M (correspondingly, 2.93%-23.2%) were added to the initial nutrient medium. In order to select strains, active xylanase producers they were cultivated under submerged fermentation in the following composition of basal liquid medium, in %: soy bean flour – 3.0; Na_2HPO_4 – 1.5; NH_4NO_3 – 0.2;

Table. Xylanase activities in cultural filtrates of mesophilic and extremophilic mycelial fungi strains

#	Culture	Xylanase activity U/ml	Characterization
1	*Aspergillus niger* T 11	18.8	Thermo- and alkalitolerant
2	*Aspergillus niger* A 82	17.5	Thermotolerant, moderate halophile
3	*Aspergillus niger* J 3-6	16.0	Thermotolerant
4	*Aspergillus niger* B 19	15.0	Phsycrotolerant
5	*Aspergillus niger* G 313	17.0	Mesophile
6	*Aspergillus terreus* X 47	16.8	Thermotolerant
7	*Aspergillus versicolor* T 16	17.5	Thermophile
8	*Aspergillus versicolor* D-1	17.5	Thermophile
9	*Aspergillus wentii* D-1	20.0	Thermophile
10	*Chaetomium thermophile* D1	10.0	Thermophile
11	*Penicillium canescence* I-85	24.0	Acidotolerant
12	*Penicillium canescence* AEM 85	52.0	Acidotolerant
13	*Penicillium canescence* TK-2	31.0	Acidotolerant
14	*Penicillium* sp. N p-4	10.0	Alkalitolerant
15	*Penicillium* sp. G-17	20.0	Mesophile
16	*Sporotrichum pulverulentum*	16.0	Thermophile
17	*Sporothrichum pulverulentum* D-1	9.5	Thermophile
18	*Sporotrichum pulverulentum* S 7	6.0	Thermotolerant
19	*Trichoderma viride* X 25	5.2	Mesophile
20	*Trichoderma lignorum* X 33	6.0	Mesophile
21	*Trichoderma viride* Ts-2	5.2	Alkalitolerant

KCl – 0.05; $MgSO_4$ – 0.015. (pH-4.5). The 10-day conidia culture suspension served as the cultivation material.

Xylanase production under submerged fermentation was carried out in 250 ml Erlenmeyer flasks on temperature-controlled rotary shaker (180-200 rpm), as required for each strain temperature (28-45^{0}C). The amount of nutrient media equaled to 50 ml. Cultivation of the strains was conducted for 70-76 h, then the medium was filtered. The filtrate was centrifuged at 4000 rot/min speed for 5 min. The clear supernatant was used for the determination of activity as a source of xylanase.

Xylanase activity was determined by mixing 0.9 ml of 1% birch wood xylan (prepared in 0.05 M Na-citrate buffer, pH 5.3) with 0.1 ml of suitably diluted enzyme solution and the mixture was incubated at 50°C for 5 min [6]. The reaction was stopped by addition of 1.5 ml of 3,5-dinitrosalicylic acid (DNS) and the content was boiled for 5 min [7]. After cooling, the color developed was measured at 540 nm. The amount of reducing sugar liberated was quantified using xylose as standard. One unit of xylanase is defined as the amount of enzyme that liberates 1 mmol of xylose equivalents per minute under the assay conditions.

Results and Discussion

In order to choose active producers of xylanase, the screening of mycelial fungi strains under submerged cultivation conditions was carried out. The studies revealed that active xylanase producers were found mainly among the representatives of the following genera: *Aspergillus, Penicillium, Sporotrichum* and

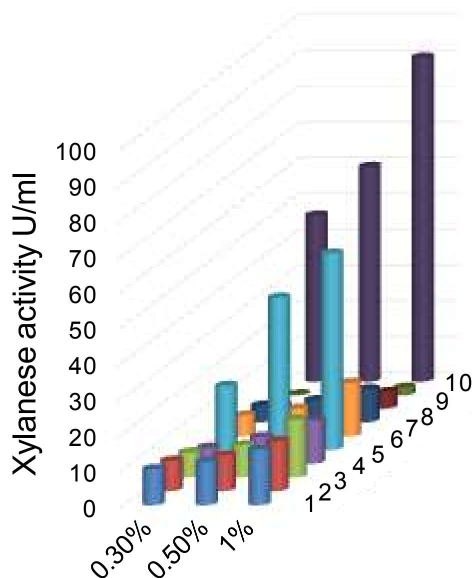

Diagram. Effect of different carbon sources on xylanase production by *Penicillium canescens* AEM 85S 1-glucose, 2-lactose, 3-mannitol, 4-rhamnose, 5-sorbitol, 6-maltose, 7-mannose, 8-fructose, 9-dulcitol, 10-xylan.

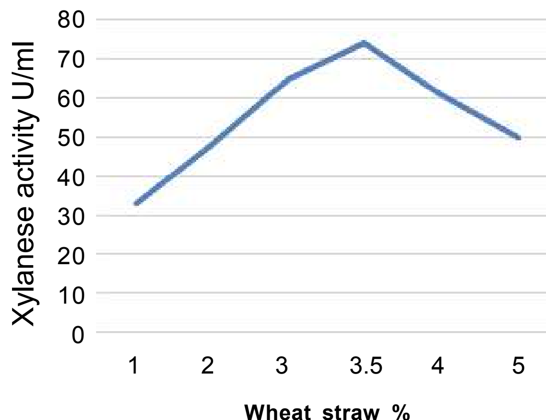

Fig. Effect of different concentrations of wheat straw on xylanase production by P. canescence AEM 85.

Trichoderma (Table 1). Finally, *Penicillium canescence,* AEM 85 strain as the most active producer of xylanase was chosen for further experiments and application.

At industrial level of enzyme or any other microbial metabolite production the cost of carbon source plays major role in the economic efficiency of the whole process.

Fermentaton of *P. canescence* AEM 85 for the production of xylanase in the basal medium supplemented with different concentrations of carbon sources (0.3%-1%), in particular: glucose, lactose mannitol, rhamnose, sorbitol, maltose, mannose, fructose, dulcitol, xylan, galactose.

For this selectively chosen strain it was determined that the xylanase biosynthesis is induced by adding in nutrient medium the substrate of xylan. The results shown in Diagram 1 indicate the inducible nature of enzyme production by *P.canescence* AEM 85 while a very low xylanase production in the medium containing maltose, lactose, mannitol, rhamnose, sorbitol, mannose, fructose, dulcitol, galactose, respectively. In the medium containing xylan, the amount of xylanase activity reached 90.5 U/ml.

The high xylan content in some wastes like corn, cobs, and wheat bran makes them accessible and cheap source as substrates and inducers. Optimization of plant sources and cultural conditions for xylanase enzyme production was carried out by adding in the basal nutrient medium substituting soy bean flour with the different agricultural and industrial wastes in 1%-5%,: wheat straw, citrus residue, apple residue, tomatoes residue, maize residue, tea residue, sawdust.

The obtained results show that the highest yield of xylanase was 74 U/ml containing 3.5 % of wheat straw. An increase of the concentration for more than 4% resulted in decrease of xylanase activity (Fig. 1).

The effect of nitrogen source on xylanase production was tested by adding different concentrations of various inorganic ($NaNO_3$, KNO_3, NH_4NO_3) and organic (yeast extract, peptone, urea) nitrogen sources.

Among the various inorganic and organic nitrogen sources tested, 0.24 % of $NaNO_3$ was the best to induce xylanase activity. Yeast extract and peptone also effectively induce the enzyme activity. The other nitrogen compounds tested were less effective.

On the basis of this study, the following cheap

composition of the nutrient media for submerged fermentation by *P. canescence* AEM 85 has been established in %: wheat straw- 3,5 ; $NaNO_3$-0,24, KCL-0,05, Na_2HPO_4 $12H_2O$-1,5. $MgSO_4$- 0.015,(pH-4.5).

According to the obtained results, depending on the optimization in nutrient medium components the activity of enzyme produced by *P. canescene* AEM 85 increased above 40%.

მიკრობიოლოგია

სამრეწველო დანიშნულების ქსილანაზას პროდუცენტი შტამის სელექცია

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ბუნებრივი ბიოპოლიმერების ფერმენტული გარდაქმნის სამრეწველო პროცესში სულ უფრო და უფრო მეტ მნიშვნელობას იძენს ჰემიცელულაზის დამშლელი ფერმენტების პროდუცენტი მიკროსკოპული სოკოების სკრინინგი. ჩატარებულია ქსილანაზას პროდუცენტი მიკროსკოპული სოკოების სელექცია სიღრმული კულტივირების პირობებში. სკრინინგის საფუძველზე საქართველოს სხვადასხვა ნიადაგობრივ-კლიმატური ზონებიდან გამოყოფილია 225 ექსტრემოფილი შტამი, რომელთაგან 21 ქსილანაზას პროდუცენტია. შერჩეული ქსილანაზას პროდუცენტებიდან 5 შტამი გამოირჩევა აღნიშნული ფერმენტის მაღალი აქტივობით. ქსილანაზას პროდუცენტებს შორის განსაკუთრებით მაღალი აქტივობებით ხასიათდებოდნენ *Aspergillus*-ის, *Penicillium*-ის, *Sporotrichum*-ის *და* *Trichoderma*-ს გვარის წარმომადგენლები. მაქსიმალური ქსილანაზური აქტივობა (52 ერთ/მლ) აღმოაჩნდა შტამს *Penicillium canescence* AEM 85. ჩატარებულია სიღრმული კულტივირებისათვის ნახშირბადისა და აზოტის წყაროების ოპტიმიზაცია. დადგენილია, რომ ქსილანაზას სინთეზი დამოკიდებულია საკვები არის შემადგენლობის ოპტიმიზაციაზე, რის შედეგადაც *P. canescene* AEM 85-ის ქსილანაზას სინთეზის უნარი 40%-ზე მეტით გაიზარდა.

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Received July, 2015