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Biochemistry

Production of Pectolytic Enzymes by Microscopic Fungi *Mucor* sp. 7 and *Monilia* sp. 10

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ABSTRACT. Pectolytic enzyme producers active and thermostable cultures of microscopic fungi *Mucor* sp. 7 and *Monilia* sp. 10 were obtained. Some physico-chemical properties of their pectinase and polygalacturonase were studied. Temperature and pH optimums of activity of the enzymes were determined. Both cultures reveal thermostability at 60°C and maintain their pectolytic activity in the wide range of pH (pH 4.0 - pH 8.0). It has been observed that biosynthesis of pectolytic enzymes by both cultures is inducible and both milk whey and pectin are effective inductors. *© 2009 Bull. Georg. Natl. Acad. Sci.*

Key words: *pectolytic enzymes, pectinase, polygalacturonase, mycelial fungi.*

Enzymes of microbial origin capable of hydrolyzing pectin are used in berry, fruit and vegetable processing and in juice settling [1,2]. Depending on the composition of the enzyme preparation completely settled and containing pulp products could be obtained .

Pectinases participate in various physiologic processes occurring in plants, particularly in fruit ripening [3]. Pectolytic enzymes of microorganisms play an important part in plant pathogenesis [4]. Pectinases are prospective in obtaining hydrolase-containing binds, with immunomodulating activity in obtaining protoplasts in cell engineering, plant selection and also in bleaching juices and wine [2-4].

Pectinases are synthesized by high plants, bacteria, yeast and mycelial fungi [5-7]. In order to obtain pectolytic enzymes it is necessary to use mycelial fungi the characteristic peculiarity of which is the production of wide range extracellular pectinases [8].

The present work is a comparative study of pectolytic enzymes produced by mycelial fungi *Mucor* sp. 7 and *Monilia* sp. 10.

Use was made of the above-mentioned filtrates of cultural liquid – *Mucor* sp. 7 and *Monilia* sp. 10 [9]. Initially the strains were grown on wort-agar within 10-15 days at 30°C.

Deep cultivation of strain was done on mineral medium of the following composition (gr/l): KH_2PO_4-3.0; $(NH_4)_2SO_4$-5.0; $MgSO_47H_2O$-0.3. 1% beet pectin and milk whey (food manufacturing waste) as carbon source were used. Initial pH values for the medium with pectin and with milk whey were pH 5.0 and pH 8.0 respectively.

The strains were grown in 750 ml flasks on a shaker (200-220t/m) for 96 h at 40°C. Amount of nutrient in flasks was 100 ml. Sowing of nutrient medium was done by 2% conidial suspension of surface strain cultures.

Dry biomass weight was determined by centrifugation on a centrifuge K-23 at 5000g for 10 min. which then was dried to constant weight at 105°C.

Pectolytic activity was determined in 0.05 M citrate buffer pH 5.0 temp. 50°C. 1% Pectin and polygalacturonic acid (pH 5.0) were used as a substrate. Reduced sugar content was estimated by the Samnera

Table 1

Effect of a carbon source and pH on pectolytic enzyme biosynthesis by microscopic fungi

	Mucor sp. 7					Monilia sp. 10				
Carbon source	Biomass gr/l	Final pH	Protein mg/ml	Pectinase U/ml	Polygalacturonase U/ml	Biomass gr/l	Final pH	Protein mg/ml	Pectinase U/ml	Polygalacturonase U/ml
Milk whey (pH 5.0)	1.7	6.8	0.56	15.2	16.5	1.4	6.6	0.7	9.5	11.0
Milk whey (pH 8.0)	1.6	7.8	1.09	16.5	17.7	1.7	7.1	0.9	10.1	12.2
Pectin (pH 5.0)	1.5	4.4	0.80	18.0	18.9	1.5	3.4	1.2	9.8	10.8
Pectin (pH 8.0)	1.6	7.5	1.0	18.8	19.2	1.6	7.4	1.2	10.5	12.0

[10] method. The activity when the enzyme hydrolyzed 1mkrmol of substrate glucoside binds in a minute at 50°C was considered per unit of enzyme activity.

Protein was determined by the modified method of Bradford [11].

To determine the temperature optimum of pectolytic enzyme activity, it was measured from 20° to 80°C with 10°C intervals. pH optimum of pectolytic enzyme activity was determined by measuring enzyme activity within pH 3.0 - 8.0 with interval 0.5. For this purpose citrate buffer and tris-HCl buffer were used. Substrates used in the work: polygalacturonic acid, galacturonic acid and citrus fruit pectin - produced by the firm "Serva"(Germany).

The data presented below were obtained as a result of three tests, each of them being repeated three times.

At the first stage of our study screening of more than 50 fungi strains isolated from various regions of Georgia was carried out and as the result 5 strains with pectolytic activity were selected. At the following stage the selected strains were grown on a liquid medium with milk whey. Pectolytic activity of the strains was 12-18 U/ml: *Mucor* sp. 7 - 12 U/ml, *Monilia* sp. 10 - 17 U/ml, *Monilia* sp. 21 – 16 U/ml, *Penicillium sp. 35* - 15U/ml, *Aspergillus* sp. 07 – 18 U/ml.

On the basis of the obtained data comparatively rare strains *Mucor* sp. 7 and *Monilia* sp. 10 were selected as producers. We have shown earlier that strains with high pectolytic activity grow well at high pH values on a liquid medium with milk whey and beet pectin.

As is known from the data, beet pectin [12] and milk whey have maximum inducing effect on pectolytic enzyme biosynthesis. Therefore at the following stage the selected strains were grown at various pH values on a liquid medium with milk whey and beet pectin. The results are shown in Table 1.

We have shown that *Mucor sp. 7* and *Monilia* sp. 10 induce both pectinase and polygalacturonase both in the medium with milk whey and with beet pectin.

Temperature optimums of pectolytic enzyme activity were determined at the further stage of our studies.

To this end, the activities of pectolytic enzymes were measured in incubating medium with intervals from 20° to 80°C by a standard method and were expressed in percentage. The results are presented in Fig 1.

From the presented data it is obvious that despite being thermotolerant temperature optimums of fungi cultures differ. For example: temperature optimum for the culture *Monilia sp.* 10 pectinase activity is 40°C, for *Mucor sp.* 7 - 50°C. It also should be mentioned that temperature optimums of pectinases and polygalacturonases of both cultures coincide and are equal to 40°C and 50°C, respectively.

Other authors data also prove that pectolytic fungi enzymes reveal maximum of their activity at 50°C [14]. Thus, it can be concluded that all isolated microscopic fungi enzymes are quite thermostable as far as at 60°C substrate depolymerization reduces only by 30%.

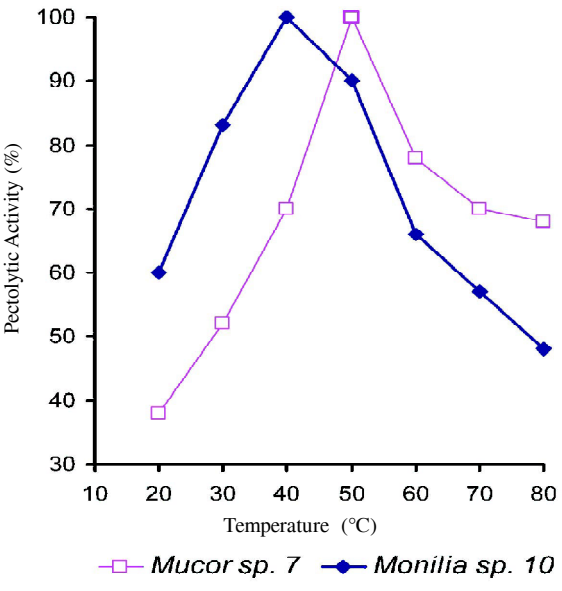

Fig. 1. Temperature optimum of pectolytic enzymes

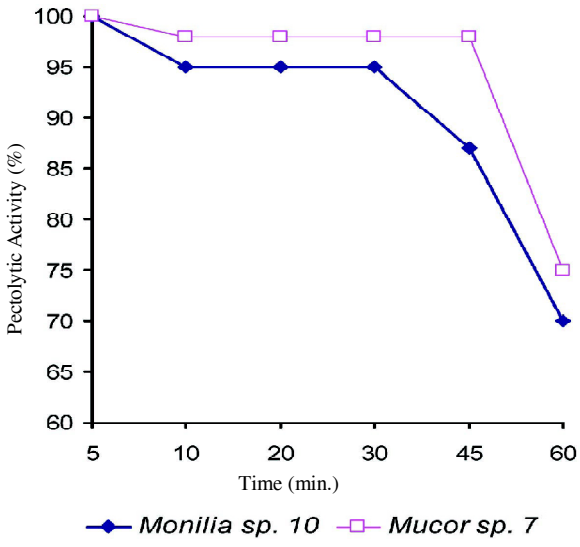

Fig. 2. Thermostability of pectolytic enzymes from the cultures *Mucor sp. 7* and *Monilia sp. 10*

The effect of temperature on the activity and stability of pectolytic enzymes was studied. At 50°C enzymes completely maintained their activity for 20 h, at 60°C - for 30 min. 2 hours later enzyme activity went down to 50%. At 70°C for 30 minutes enzymes lost 50% of activity. At 80°C for the same period of time enzymes were almost completely inactivated. At room temperature enzymes maintained their activity without any loss for 7 hours. These data demonstrate the thermostability of pectolytic enzymes. The results of studies of thermostability of the mentioned cultures producers of pectolytic enzymes at 60°C in terms of time are shown in Fig. 2.

According to the presented data pectolytic enzymes of *Mucor* sp. 7 and *Monilia* sp. 10 within 30 min. did not lose activity, yet an hour later enzyme activity went down to 71% and 75%, respectively. Thus, the studies carried out resulted in obtaining active and thermostable producers of pectolytic enzymes – the cultures of *Mucor* sp. 7 and *Monilia* sp. 10.

The results of estimation of pH values appeared quite interesting, i.e. for the fungus *Mucor* sp. 7 maximum pectinase activity was observed at pH 4.0, then at pH 5.0 it reduced to 52% and stayed unchanged up to pH 8.0. In the case of the fungus *Monilia* sp. 10 maximum pectinase activity was observed at pH 5.0 and by pH 8.0 it reduced only by 12%. Concerning polygalacturonase activity, for the fungus *Mucor* sp. 7 maximum enzyme activity was observed at pH 5.5 and by pH 8.0 it went down only by 10%. The results are shown in Fig.3.

As for the activity of polygalacturonase for the fungus *Monilia* sp. 10. maximum activity of the enzyme is observed at pH 5.0 and by pH 8.0 it goes down almost by 40%. The results are shown in Fig. 4.

The results of tests have shown that both fungi cultures demonstrate wide scale of maximum pectolytic activity in a wide range of pH (4.0 - 8.0).

Thus, active and thermostable producers of pectolytic enzymes have been obtained from the cultures *Mucor* sp. 7 and *Monilia* sp. 10. It has also been ascertained that both fungi cultures demonstrate maximum pectolytic activity within a wide range of pH (4.0 - 8.0).The results of the work performed have shown that pectolytic enzymes of mycelial fungi *Mucor* sp.7 *and Monilia* sp 10 are inducible and both beet pectin and milk whey were found to be the most effective inductors for the cultures.

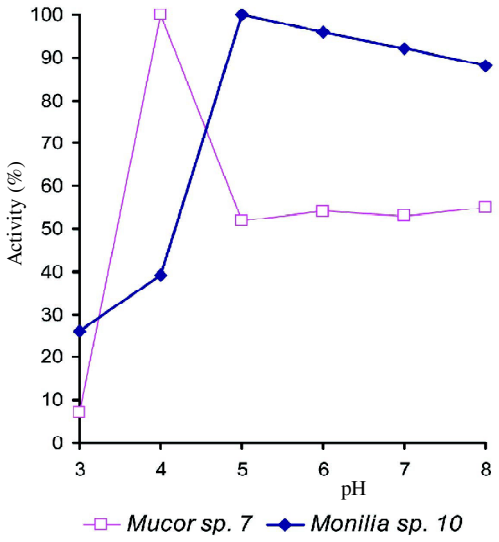

Fig.3. pH influence on the pectolytic activity of *Mucor sp. 7* and *Monilia sp. 10*

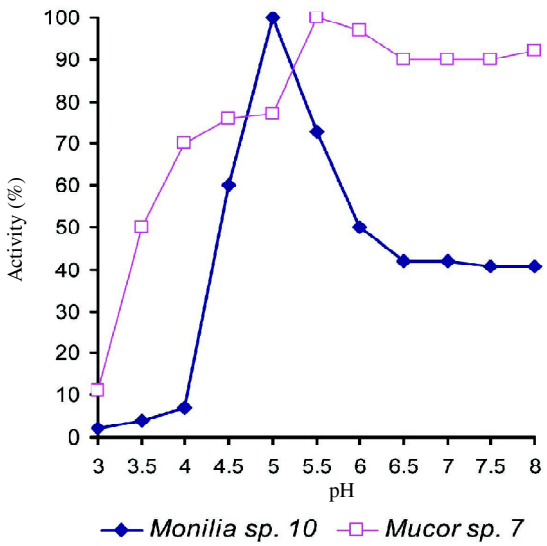

Fig.4. pH optimum of polygalacturonase from cultures *Mucor sp. 7* and *Monilia sp. 10*

ბიოქიმია

მიკროსკოპული სოკოების *Mucor* sp. 7-ისა და *Monilia* sp. 10-ის პექტოლიზური ფერმენტები

ა. წერეთელი*, ლ. დაუშვილი*, თ. ბუაჩიძე**, ე. კვესიტაძე*, ნ. ბუცხრიკიძე**

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მიღებულია პექტოლიზური ფერმენტების პროდუცენტი მიკროსკოპული სოკოების აქტიური და თერმოსტაბილური კულტურები *Mucor* sp. 7 და *Monilia* sp. 10. შესწავლილია მათი პექტინაზას და პოლიგალაქტურონაზას ზოგიერთი ფიზიკურ-ქიმიური თვისება. დადგენილია ფერმენტების აქტივობის ტემპერატურული და pH-ოპტიმუმები. დადგენილია, რომ ორივე კულტურა 60°C-ზე საკმაო თერმოსტაბილურობას ამჟღავნებს და პექტოლიზურ აქტივობას ინარჩუნებს pH-ის ფართო დიაპაზონში (pH 4.0 - pH 8.0). დადგენილია, რომ ორივე კულტურის მიერ პექტოლიზური ფერმენტების ბიოსინთეზი ინდუცირებადია და რძის შრატი და პექტინი ეფექტურ ინდუქტორებს წარმოადგენენ.

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