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EXPERIMENTAL ARTICLES

# Isoelectrophoretic Characterization of Extracellular Polygalacturonases of Various *Aspergillus alliaceus* Strains

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**Abstract**—The composition of pectin hydrolase complexes produced by various *Aspergillus alliaceus* strains was studied under the conditions of induction, catabolite repression, or constitutive synthesis. The strains were found similar in terms of the polygalacturonase spectrum and different with regard to the levels of endo- and exoenzyme activities. The analysis of the zymograms of inducible polygalacturonases revealed that all tested cultures contained at least 24 molecular forms of polygalacturonase. Taking into account only the three molecular forms typical of all analyzed strains of *A. alliaceus* with pI values of 5.7, 5.9, and 6.3, one can use the spectrum of constitutive, catabolite repression-resistant polygalacturonases as an additional taxonomic species criterion.

*Key words: Aspergillus alliaceus*, strains, polygalacturonases, biosynthesis, induction, constitutive synthesis, catabolite repression, multiple molecular forms, zymograms, identification

Phytopathogenic and saprophytic fungi in vivo and in vitro produce extracellular enzymes catalyzing the degradation of plant polysaccharides including pectin substances [1–5]. The pectinolytic complexes synthesized by fungi contain enzymes with different modes and sites of action and specificity with respect to substrate structure [6, 7]. Fungal polygalacturonases, like other pectinolytic enzymes, exist in multiple molecular forms differing in electrophoretic mobility, molecular mass, substrate specificity, and a number of other properties [8–10].

It was established earlier that the mycelial fungus *Aspergillus alliaceus* BIM-83 is a highly active producer of polygalacturonase complex, which consists of multiple molecular forms of endo- and exopolygalcturonases whose synthesis involves different mechanisms [11–14]. It was also shown that polygalacturonase (PG) formation is characteristic of 11 *A. alliaceus* strains isolated from natural habitats [15]. The goal of this work was (i) to investigate the composition of the pectin hydrolase complexes produced by the above *A. alliaceus* strains under induction, catabolite repression, and constitutive synthesis and (ii) to carry out a comparative analysis of the enzyme isoelecrtophoregrams obtained.

## MATERIALS AND METHODS

In this study we used 11 strains of *Aspergillus alliaceus* Thom et Church obtained from the microbial culture collections of the Institute of Microbiology (National Academy of Sciences, Belarus), the Zabolotnyi Institute of Microbiology and Virology (National Academy of Sciences, Ukraine), the Institute of Biochemistry and Physiology of Microorganisms (Russian Academy of Sciences), and the Khar'kov Branch of the Research Institute of Beverages and Mineral Waters (Ukraine).

The strains of *A. alliaceus* were cultivated in 250-ml Erlenmeyer flasks containing 50 ml of medium on a shaker (180–200 rpm) at 24–26°C for 96 h. An aqueous suspension of the spores of 14-day-old cultures grown on potato–glucose agar at 24–26°C served as inoculum ($1 \times 10^7$ spores per 50 ml of medium).

The medium for cultivating fungi contained (%): malt extract, 5.0; $(NH_4)_2SO_4$, 0.7; $KH_2PO_4$, 0.1; $MgSO_4 \cdot 7H_2O$, 0.05; KCl, 0.05; and $Fe_2(SO_4)_3 \cdot 7H_2O$, 0.001. The initial pH value was 4.8–5.0. Apple pectin (1%), glucose (1%), or apple pectin (0.5%) + glucose (0.5%) were used as the carbon source.

Fungal biomass was separated by filtration, dried at 105°C, and weighed.

Total pectinolytic activity (PA) was determined interferometrically, endopolygalacturonase (endoPG) activity—viscosymetrically, and exopolygalacturonase (exoPG) activity—titrometrically using a 1% solution of beet pectin (methoxylation degree of 38%) as the substrate [16, 17]. Enzyme activity was expressed in U/ml of culture fluid, U/mg of dry biomass (producing capacity of mycelium), and U/mg of protein (specific activity).

To perform analytical isoelectrofocusing and native electrophoresis of the extracellular pectinases produced by various *A. alliaceus* strains, the filtrates of the cul-

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**Table 1.** Growth of various *A. alliaceus* strains on the medium with apple pectin. Pectin hydrolase synthesis

| Strain | Final pH value | Biomass, mg/ml | Protein, μg/ml | PA | | | EndoPG | | | ExoPG | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | U/ml | U/mg of biomass | U/μg of protein | U/ml | U/mg of biomass | U/μg of protein | U/ml | U/mg of biomass | U/μg of protein |
| BIM-83 | 2.8 | 3.24 | 22 | 1.97 | 0.68 | 0.090 | 2362 | 729.0 | 107.36 | 11.17 | 3.45 | 0.508 |
| 900127 | 3.1 | 2.82 | 23 | 1.90 | 0.67 | 0.083 | 125 | 44.3 | 5.43 | 5.47 | 1.94 | 0.238 |
| 900128 | 3.2 | 2.90 | 17 | 1.14 | 0.39 | 0.067 | 131 | 45.3 | 7.74 | 4.10 | 1.41 | 0.241 |
| 900129 | 3.2 | 2.60 | 24 | 1.22 | 0.47 | 0.051 | 200 | 76.9 | 8.33 | 6.16 | 2.37 | 0.257 |
| 900130 | 3.2 | 2.58 | 21 | 0.74 | 0.29 | 0.035 | 260 | 100.6 | 12.37 | 4.10 | 1.59 | 0.195 |
| 900131 | 3.2 | 2.74 | 17 | 1.50 | 0.55 | 0.032 | 227 | 82.9 | 13.36 | 2.74 | 1.00 | 0.161 |
| 900132 | 2.9 | 2.92 | 20 | 1.41 | 0.48 | 0.071 | 598 | 204.8 | 29.90 | 4.79 | 1.64 | 0.240 |
| 497 | 3.1 | 3.40 | 24 | 1.93 | 0.57 | 0.080 | 468 | 137.5 | 19.48 | 4.79 | 1.41 | 0.200 |
| VKM F-764 | 3.0 | 3.00 | 25 | 1.73 | 0.58 | 0.069 | 555 | 185.2 | 22.22 | 6.84 | 2.28 | 0.274 |
| VKM F-2248 | 3.1 | 3.00 | 30 | 1.15 | 0.38 | 0.038 | 533 | 177.8 | 17.78 | 4.10 | 1.37 | 0.137 |
| VKM F-2994 | 3.2 | 2.90 | 25 | 0.44 | 0.15 | 0.018 | 282 | 97.1 | 11.27 | 3.42 | 1.18 | 0.137 |

ture fluids were concentrated to 1/10 of the starting volume with an RVO-64 rotation vacuum evaporator (Czechia) and centrifugated. Desalinization of the supernatant was carried out on a Molselect G-25 column (Reanal, Hungary). The fractions exhibiting pectinolytic activity were recombined and used to determine the protein content and to separate the molecular forms of the enzyme.

Protein separation on 5% PAAG plates (pH 3.5–9.5, Serva, Germany) was carried out with a Multiphor device (LKB, Sweden) for 3–3.5 h at a constant power of 12 W. The initial current and voltage were 40 mA and 250 V, respectively. 0.5 M NaOH and 0.5 M acetic acid served as electrode buffers. Native electrophoresis of enzyme proteins was done according to Laemmli [18].

PG location after isoelectrofocusing and electrophoresis was determined directly in gels. For this purpose, gels prewashed with distilled water and 0.02 M acetate buffer (pH 4.8) were incubated in a 1.2% solution of beet pectin in 0.02 M acetate buffer (pH 4.8) at 35–40°C for 20–30 min. The gels were thereupon washed with distilled water and developed with a 0.05% solution of ruthenium red. The pH gradient in the gel was determined using pI markers (pH 3–10, Pharmacia, Sweden). The isoelectric point values of the major PG forms were measured in the gel with microelectrodes.

Malt seedling extract was prepared according to Fertman and Girs [19]. Proteins were determined by the Bradford method [20]. The pH values were measured potentiometrically. The data presented in this paper were the means of 3–5 experiments; each experiment was run in three replicates.

## RESULTS AND DISCUSSION

Comparative studies were conducted on PG complexes produced by various *Aspergillus alliaceus* strains as a result of inducible, catabolite-repressible, and constitutive enzyme synthesis. Growing the fungi in medium with pectin as the carbon source enabled us to determine the whole PG spectrum, including inducible and constitutive enzyme forms. Using a complex carbon source (pectin + glucose) resulted in the production of the inducible and constitutive enzymes whose synthesis is not repressed by a catabolite. Employing a medium with glucose as the sole carbon source made it possible to detect a constitutive PG whose formation is not (or only insignificantly) catabolite-repressible.

From the data of Tables 1–3, it follows that medium acidity increased towards the end of the cultivation period. The pH values were 2.8–3.2, 2.0–2.3, and 2.4–3.1 with pectin, glucose, and their combination, respectively. Less biomass per 1 ml of culture fluid accumulated with pectin than with glucose or glucose + pectin. However, extracellular protein production by the fungi was maximum (17–30 μg/ml) with pectin.

Inducible enzyme synthesis in all tested *A. alliaceus* strains yielded polygalacturonase complexes with different activities of their components (Table 1). The total pectinolytic activity of the least active strain, VKM F-2994, was only 0.44 U/ml, i.e., 4.5 times less than that of the industrial strain BIM-83 (1.97 U/ml). However, strains 497 and 900127 virtually attained the level of strain BIM-83 in terms of pectin hydrolase synthesis and the producing capacity of mycelium. In addition, the fungal enzyme complexes differed in the endoPG–exoPG ratio. This ratio was minimum (28) with *A. alliaceus* 900127 and maximum (211) with strain BIM-83.

Supplementing the medium with 0.5% glucose in addition to apple pectin resulted in decreasing exoPG

**Table 2.** Growth of various *A. alliaceus* strains on the medium with apple pectin and glucose. Pectin hydrolase synthesis

| Strain | Final pH value | Biomass, mg/ml | Protein, μg/ml | PA | | | EndoPG | | | ExoPG | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | U/ml | U/mg of biomass | U/μg of protein | U/ml | U/mg of biomass | U/μg of protein | U/ml | U/mg of biomass | U/μg of protein |
| BIM-83 | 2.4 | 3.96 | 8 | 0.130 | 0.033 | 0.016 | 48.8 | 12.32 | 6.098 | 1.37 | 0.35 | 0.171 |
| 900127 | 2.9 | 5.27 | 14 | 0.450 | 0.085 | 0.032 | 188.7 | 35.81 | 13.480 | 16.50 | 3.13 | 1.179 |
| 900128 | 2.9 | 4.86 | 12 | 0.450 | 0.093 | 0.038 | 379.5 | 78.08 | 31.630 | 14.36 | 2.95 | 1.197 |
| 900129 | 2.9 | 5.10 | 10 | 0.380 | 0.075 | 0.038 | 714.8 | 140.16 | 71.480 | 15.39 | 3.02 | 1.539 |
| 900130 | 3.0 | 5.26 | 13 | 0.410 | 0.078 | 0.032 | 405.0 | 77.00 | 31.150 | 13.40 | 2.55 | 1.031 |
| 900131 | 3.1 | 5.06 | 9 | 0.200 | 0.040 | 0.022 | 273.0 | 53.95 | 30.330 | 9.20 | 1.82 | 1.022 |
| 900132 | 3.0 | 5.14 | 15 | 0.410 | 0.080 | 0.027 | 269.8 | 52.49 | 17.990 | 16.42 | 3.19 | 1.095 |
| 497 | 2.9 | 4.95 | 15 | 1.330 | 0.269 | 0.089 | 952.3 | 192.38 | 63.490 | 21.54 | 4.35 | 1.436 |
| VKM F-2248 | 3.0 | 4.88 | 16 | 1.290 | 0.264 | 0.081 | 810.8 | 166.15 | 50.680 | 22.57 | 4.63 | 1.411 |
| VKM F-2248 | 3.0 | 4.68 | 16 | 0.450 | 0.096 | 0.028 | 450.3 | 96.22 | 28.140 | 17.44 | 3.73 | 1.090 |
| VKM F-2994 | 2.9 | 5.70 | 16 | 0.350 | 0.061 | 0.022 | 125.5 | 22.02 | 7.840 | 15.39 | 2.70 | 0.962 |

**Table 3.** Growth of various *A. alliaceus* strains on the medium with glucose. Pectin hydrolase synthesis

| Strain | Final pH value | Biomass, mg/ml | Protein, μg/ml | PA | | | EndoPG | | | ExoPG | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | U/ml | U/mg of biomass | U/μg of protein | U/ml | U/mg of biomass | U/μg of protein | U/ml | U/mg of biomass | U/μg of protein |
| BIM-83 | 2.3 | 3.08 | 3.5 | 0.004 | 0.001 | 0.0011 | 5.26 | 1.71 | 1.503 | 0.27 | 0.090 | 0.077 |
| 900127 | 2.3 | 4.84 | 7.5 | 0.011 | 0.002 | 0.0015 | 5.30 | 1.16 | 0.707 | 0.82 | 0.169 | 0.109 |
| 900128 | 2.1 | 4.56 | 4.0 | 0.015 | 0.003 | 0.0038 | 5.30 | 1.16 | 1.325 | 0.55 | 0.121 | 0.138 |
| 900129 | 2.0 | 5.30 | 14.0 | 0.019 | 0.004 | 0.0014 | 10.95 | 2.07 | 0.782 | 1.03 | 0.194 | 0.074 |
| 900130 | 2.1 | 4.96 | 6.0 | 0.016 | 0.003 | 0.0027 | 9.41 | 1.90 | 1.568 | 0.48 | 0.097 | 0.080 |
| 900131 | 2.3 | 5.26 | 6.0 | 0.015 | 0.003 | 0.0025 | 10.25 | 1.71 | 1.708 | 0.62 | 0.118 | 0.103 |
| 900132 | 2.2 | 6.20 | 5.0 | 0.010 | 0.002 | 0.0020 | 10.38 | 1.67 | 2.076 | 0.34 | 0.055 | 0.068 |
| 497 | 2.1 | 3.36 | 8.0 | 0.011 | 0.003 | 0.0014 | 9.87 | 2.94 | 1.234 | 0.21 | 0.063 | 0.026 |
| VKM F-764 | 2.1 | 4.90 | 7.0 | 0.010 | 0.002 | 0.0014 | 12.19 | 2.49 | 1.741 | 0.68 | 0.139 | 0.097 |
| VKM F-2248 | 2.2 | 4.38 | 16.0 | 0.015 | 0.003 | 0.0009 | 28.85 | 6.59 | 1.803 | 1.64 | 0.374 | 0.103 |
| VKM F-2994 | 2.1 | 5.82 | 15.0 | 0.014 | 0.002 | 0.0009 | 10.25 | 1.76 | 0.683 | 0.34 | 0.058 | 0.023 |

synthesis by all tested strains (Table 2). The repressive effect of glucose was minimum (a 20.5% decrease in pectinolytic activity) and maximum (93.4%) with *A. alliaceus* VKM F-2994 and strain BIM-83, respectively.

Cultivation of all tested fungi on media with glucose minimized the synthesis of extracellular pectinases, and the pectinolytic activity in strains BIM-83 and VKM F-2994 was only 0.2 and 3.2% of that observed on media with pectin (Table 3). Total pectinolytic activity values ranged from 0.019 to 0.004 U/ml. Maximum endoPG activity was found in *A. alliaceus* VKM F-2248, whereas strains 900129 and VKM F-2248 exhibited maximum exoPG activity.

The heterogeneity of the PG complexes produced by the *A. alliaceus* strains constitutively and upon induction was evaluated using native electrophoresis and isoelectrofocusing in PAAG with different pH gradient profiles, with subsequent staining of the gels to determine proteins and pectinolytic enzymes. The results obtained demonstrated that electrophoretically separated fungal PG formed one continuous zone whose size depended on the protein content (ranging from 5 to 25 μg) in the samples. Therefore, the pectinolytic complexes of the tested fungi were composed of proteins with identical or similar molecular weights.

Isoelectrophoretic separation of the PG of the tested *A. alliaceus* strains with the pH range of 3.5–9.5 indicated that they concentrated in the anode portion of the gel. Therefore, it was impossible to determine the precise amount of the PG forms synthesized by the fungi. Employing PAAG with 1% ampholite, pH 2.0–4.5, 1%

ampholite, pH 4.0–6.5, and 0.5% ampholite, pH 3.5–9.5, for isoelectrofocusing, varying the compositions and concentrations of the electrode buffers, and applying the samples onto various gel parts enabled us to accurately separate the PG complex into the individual forms. With samples (5–20 μg of protein) applied near the cathode and 0.5% acetic acid and 2% ethylene diamine solutions used as cathode and anode buffers, respectively, the process was run for 3–3.5 h at 5°C.

The scheme given in Fig. 1a shows the PG spectrum secreted by the *A. alliaceus* strains on medium with apple pectin. Upon induction, the fungi produced complexes containing at least 24 components varying in their proportions. Three PG forms with isoelectric point values of 5.7, 5.9, and 6.3 prevailed in all *A. alliaceus* strains upon induction. They were the only constituents of the enzyme complexes if the fungi were cultivated with glucose as the sole carbon source (Fig. 1b). The synthesis of these PG forms was constitutive; it was not repressed by an easily metabolized carbon source.

Therefore, the results obtained suggest that (i) the PG complexes produced by the *A. alleaceus* strains isolated from various habitats are characterized by molecular heterogeneity and (ii) the synthesis of the enzyme forms is controlled by different regulatory mechanisms.

Other researchers also presented evidence that the synthesis of the molecular enzyme forms represented in the pectinolytic complexes of microorganisms is governed by different mechanisms. The phytopathogenic fungus *Sclerotinia sclerotiorum*, using pectin as a carbon source, synthesized an enzyme complex containing 9 pectinase forms, including 5 multiple molecular PG forms and 4 pectin methylesterases. Cultivating the fungus on a glucose-containing medium resulted in a decrease in the numbers of PG and pectin methylesterases to 3 and 2 molecular forms, respectively [5]. The fact that *Phomopsis cucurbitae* produced at least 15 enzyme forms with pI values of 3.7–8.6 on medium with pectin suggests the involvement of induction and catabolite repression mechanisms in the regulation of PG formation. Seven enzyme isoforms (pI 4.0, 4.2, 4.5, 7.3, 7.5, 7.8, and 8.6) were revealed with glucose and sucrose, and five isoforms (pI 4.0, 4.2, 7.5, 7.8, and 8.6), with galactose as the carbon source [2].

Recently, electrophoresis and isoelectrofocusing of intra- and extracellular enzyme proteins have been widely used in fungal taxonomy, because zymograms represent the most convenient and readily available biochemical markers of cultures. Zymograms of various dehydrogenases, superoxide dismutases, esterases, etc. [21–23] are typically used as additional diagnostic tests. It has been suggested that electrophoregrams of pectinolytic enzymes should be employed in fungal taxonomy [24]. This method helped to more accurately determine the systematic position of the representatives of the genera *Sclerotinia* [25], *Penicillium* [26], and *Armillaria* [27]. Morphologically similar *Fusarium* species were classified [28, 29], and *Gremmeniella* sp. isolates were characterized on this basis [30].

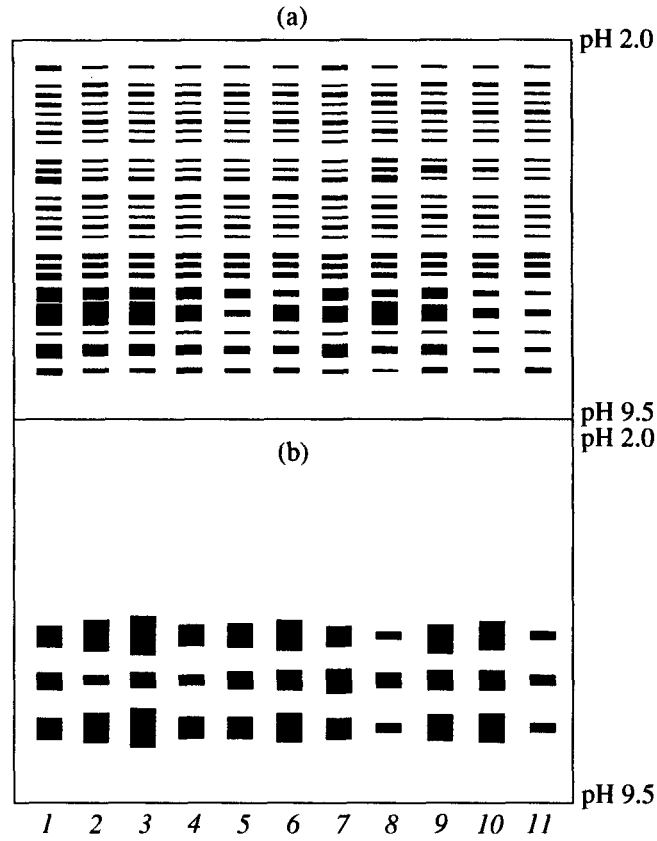


**Fig. 1.** Isoelectrophoregrams of the extracellular PG produced by various *A. alliaceus* strains on media with (a) pectin and (b) glucose. (*1*) BIM-83; (*2*) 900127; (*3*) 900128; (*4*) 900129; (*5*) 900130; (*6*) 900131; (*7*) 900132; (*8*) 497; (*9*) VKM F-764; (*10*) VKM F-2248; (*11*) VKM F-2294.

Based on the results obtained by us, the zymograms of constitutive, catabolite repression–resistant PG represent a useful supplementary taxonomic criterion of the *A. alliaceus* species. Presumably, the zymograms of extracellular constitutive, catabolite repression-resistant PG and other enzymes can be widely used for classifying fungi of diverse genera.

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## REFERENCES

1. Takasawa, T., Sagisaka, K., Yagi, K., Uchiyama, K., Aoki, A., Takaoka, K., and Yamamato, K., Polygalacturonase Isolated from the Culture of the Psychrophilic Fungus *Sclerotinia borealis, Can. J. Microbiol.*, 1997, vol. 43, pp. 417–424.
2. Zhang, J., Bruton, B.D., and Biles, C.L., Polygalacturonase Isozymes Produced by *Phomopsis cucurbitae* in

Relation to Postharvest Decay of Cantaloupe Fruit, *Phytopathology*, 1997, vol. 87, pp. 1020–1025.

3. Guevara, M.A., Gonzales-Jaen, M.T., and Estevez, P., Multiple Forms of Pectic Lyases and Polygalacturonase from *Fusarium oxysporum* f. sp. *Radicis lycopersici*: Regulation of the Synthesis by Galacturonic Acid, *Can. J. Microbiol.*, 1997, vol. 43, pp. 245–253.
4. Tobias, R.B., Conway, W.S., and Sams, C.E., Polygalacturonase Produced in Apple Tissue Decayed by *Botrytis cinerea, Biochem. Mol. Biol. Int.*, 1995, vol. 35, no. 4, pp. 813–823.
5. Riou, C., Fraissinet-Tachet, L., Freyssinet, G., and Ferve, M., Secretion of Pectic Isoenzymes by *Sclerotinia sclerotiorum, Microbiol. Lett.*, 1992, vol. 91, pp. 231–238.
6. Deuel, H. and Stutz, E., Pectic Substances and Pectic Enzymes, *Adv. Enzymol.*, 1958, vol. 20, pp. 341–382.
7. Pilnic, K.W., Rombouts, F.M., and Voragen, A.G.J., On the Classification of Pectin Depolymerases: Activity of Pectin Depolymerases on Glycol Esters of Pectate as a New Classification Criterion, *Chem. Microbiol. Technol. Lebensm.*, 1973, vol. 2, no. 4, pp. 122–128.
8. Kester, H.C. and Visser, J., Purification and Characterization of Polygalacturonases Produced by Hyphal Fungus *Aspergillus niger, Biotechnol. Appl. Chem.*, 1990, vol. 12, pp. 150–160.
9. Behere, A., Satyanarayan, V., and Padwal-Desai, S.R., Separation and Limited Characterization of Three Polygalacturonases of *Aspergillus niger, Enzyme Microbiol. Technol.*, 1993, vol. 15, no. 2, pp. 158–161.
10. Devi, N.A. and Rao, A.G.A., Fractionation, Purification, and Preliminary Characterization of Polygalacturonases Produced by *Aspergillus carbonarius, Enzyme Microb. Technol.*, 1996, vol. 18, pp. 59–65.
11. Lobanok, A.G., USSR Inventor's Certificate no. 722946, SSSR, MKI2 S12 K 1/00 (SSSR), *Byul. Izobr.*, 1980, no. 11.
12. Sapunova, L.I., Mikhailova, R.V., and Lobanok, A.G., Separation and Analysis of Polygalacturonases Produced by *Aspergillus alliaceus* on Medium with Glucose, *Prikl. Biokhim. Mikrobiol.*, 1996, vol. 32, no. 5, pp. 506–509.
13. Mikhailova, R.V., Sapunova, L.I., and Lobanok, A.G., Regulation of the Synthesis of Enzymes of the Pectin Hydrolase Complex in *Aspergillus alliaceus, Prikl. Biokhim. Mikrobiol.*, 1992, vol. 28, no. 2, pp. 249–255.
14. Mikhailova, R.V., Sapunova, L.I., and Lobanok, A.G., Three Polygalacturonases Constitutively Synthesized by *Aspergillus alliaceus, Wld. J. Microbiol. Biotechnol.*, 1995, vol. 11, no. 3, pp. 330–332.
15. Mikhailova, R.V., Sapunova, L.I., Zenkovich, I.E., Shishko, Zh.F., and Lobanok, A.G., Depolymerases of Different Strains of *Aspergillus alliaceus* Thom et Church, *Mikol. Fitopatol.*, 1995, vol. 29, no. 1, pp. 55–60.
16. Rukhlyadeva, A.P. and Korchagina, G.T., Determination of the Activity of Pectinolytic Enzymes of Fungal Origin by the Interferometric Method, *Prikl. Biokhim. Mikrobiol.*, 1973, vol. 9, no. 6, pp. 922–927.
17. Lifshits, D.B., Methods for Determination of Pectinolytic Activity of Fungal Preparations, *Unifitsirovanie metodov opredeleniya aktivnosti fermentnykh preparatov proizvodstvennogo naznacheniya* (Unification of the Methods for Determination of the Enzymatic Activity of Preparations Designed for Industrial Application), Kiev: Naukova Dumka, 1967, pp. 33–38.
18. Laemmli, U.K., Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4, *Nature* (London), 1970, vol. 227, pp. 680–685.
19. Fertman, G.N. and Girs, V.T., Amino Acid and Carbohydrate Composition of Malt Seedling Extracts, *Prikl. Biokhim. Mikrobiol.*, 1969, vol. 5, no. 5, pp. 563–566.
20. Bradford, M.M., A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing Principle of Protein-Dye Binding, *Anal. Biochem.*, 1976, vol. 72, pp. 248–254.
21. Suprun, L.M., Rybakova, I.N., Terekhova, V.A., and D'yakov, Yu.T., Investigation of the Population of the Causative Agent of Potato Phytophtorosis with the Use of Biochemical Markers, *Dokl. Vses. Akad. Skh. Nauk im. V.I. Lenina*, 1986, no. 1, pp. 16–19.
22. Lekomtseva, S.N., Chaika, M.N., and Volkova, V.T., Isoenzyme Composition of Some Specialized Forms of *Puccinia graminis* Pers, *Mikol. Fitopatol.*, 1996, vol. 30, no. 2, pp. 35–38.
23. Vaguifalvi, A. and Szecsi, A., Esterase Isozyme Variation among *Fusarium moniliforme, F. proliferatum*, and *F. subglutinans, Acta Phytopathol. Entomol. Hung.*, 1994, vol. 29, no. 3/4, pp. 231–238.
24. Cruickshank, R.N. and Wade, G.C., Detection of Pectic Enzymes in Pectin–Acrylamide Gels, *Anal. Biochem.*, 1980, vol. 107, pp. 177–181.
25. Cruickshank, R.H., Distinction between *Sclerotinia* Species by Their Pectic Zymograms, *Trans. Brit. Mycol. Soc.*, 1983, vol. 80, no. 1, pp. 117–119.
26. Cruickshank, R.N. and Pitt, J.I., Identification of Species in *Penicillium* Subgenus *Penicillium* by Enzyme Electrophoresis, *Mycologia*, 1987, vol. 79, no. 4, pp. 614–630.
27. Wahlstrom, K., Karlsson, J.O., Holdenrider, O., and Stenled, J., Pectinolytic Activity and Isozymes in European *Armillaria* Species, *Can. J. Bot.*, 1991, vol. 69, pp. 2732–2739.
28. Szecsi, A., Analysis of Pectic Enzyme Zymograms of *Fusarium* Species: I. *Fusarium lateritium* and Related Species, *J. Phytopathol.*, 1990, vol. 129, no. 1, pp. 75–83.
29. Szecsi, A., *Analysis of Pectic Enzyme Zymograms of Fusarium* Species: II. Comparison of Polygalacturonase Zymograms of *Fusarium culmorum* and *Fusarium graminearum, J. Phytopathol.*, 1990, vol. 130, no. 3, pp. 188–196.
30. Lecours, N., Totil, Sieber, T., and Petrini, O., Pectic Enzyme Patterns as a Taxonomic Tool for the Characterization of *Gremminiella* sp. Isolates, *Can. J. Bot.*, 1994, vol. 72, pp. 891–896.