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Isolation and Characterization of Extracellular Pectin Lyase from *Penicillium canescens*

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Abstract—Pectin lyase A (molecular weight 38 kD by SDS-PAGE, p*I* 6.7) was purified to homogeneity from culture broth of the mycelial fungus *Penicillium canescens* using chromatographic techniques. During genomic library screening, the gene encoding pectin lyase A from *P. canescens* (*pelA*) was isolated and sequenced, and the amino acid sequence was generated by applying the multiple alignment procedure (360 residues). A theoretical model for the three dimensional structure of the protein molecule was also proposed. Different properties of pectin lyase A were investigated: substrate specificity, pH- and temperature optimum of activity, stability under different pH and temperature conditions, and the effect of Ca^{2+} on enzyme activity. In the course of the laboratory trials, it was demonstrated that pectin lyase A from *P. canescens* could be successfully applied to production and clarification of juice.

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Pectin is a component of the middle lamella of primary cell wall of the majority of higher plant tissues [1, 2]. Pectin structure is composed of alternating "smooth" and highly branched areas. The smooth areas (homogalactouronan) represent the backbone chain of the pectin molecule, containing α-1,4-linked residues of *D*-galacturonic acid, which can be methylated at the O-6 position. Highly branched areas (rhamnogalactouronan) contain large numbers of side chains, α-1,2-linked residues of *L*-rhamnopyranose. In addition, *D*-galacturonic acid can bear β-xyloside substituent at the O-3 position. The ratio of pectin structural elements varies over a wide range and is dependent on the plant source [1-4].

The enzymes involved in degradation of pectin are widespread in nature and are produced by many plants, bacteria, and fungi [5]. One of the important components of pectinase enzyme complex is pectin lyase (EC 4.2.2.10). It catalyzes the cleavage of α-1,4-*D*-glycoside bond between methoxylated residues of pectin's galacturonic acid by means of β-elimination and formation of Δ-4,5-unsaturated product. All pectin lyases known today are endoenzymes.

Pectin lyase is the only component of pectinase complex (from those known today) able to act directly on highly etherized substrates, decreasing their viscosity (without their preliminary deetherification, which usually precedes hydrolytic cleavage of pectin). Pectin lyase enables juice clarification without destruction of volatile ester components, conferring specific fruit fragrance to the juice. It is important that the use of pectin lyases in juice production does not result in formation of

Abbreviations: MD) methylation degree; PGA) polygalacturonic acid; p*I*) isoelectric point; PL A, PL B) pectin lyases A and B, respectively; RS) reducing sugars.

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methanol. These advantages explain the growing interest of researchers in pectin lyases [6].

This work was focused on isolation and investigation of properties of extracellular pectin lyase secreted by the mycelial fungus *Penicillium canescens*, as well as on the study of potential and conditions for practical application of the enzyme.

MATERIALS AND METHODS

Enzyme preparations. This work used culture broth of fungus *P. canescens* PCA-10 from the Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences (Pushchino), as well as liquid preparations Rapidase C-80L and Rapidase Press supplied by Gist Brocades/DSM (The Netherlands), obtained from a fungal strain of *Aspergillus niger*.

Substrates. The following substrates were used for determination of enzyme activity: apple pectin, citrus pectins with methylation degree (MD) of 26, 65, 70, and 89%, and polygalacturonic acid (PGA), all purchased from Sigma (USA); red beet pectin (Reakhim, Russia).

Determination of enzyme activities. Pectin lyase activity was determined by a method based on measuring the initial rate of the accumulation of Δ-4,5-unsaturated pectin destruction products [7]. To a thermostatted (40°C) spectrophotometer cuvette containing 2.9 ml of substrate solution (0.25%) in 0.05 M sodium acetate buffer, pH 5.0, 0.1 ml of enzyme solution was added, followed by the registration of kinetics of accumulation of non-saturated reaction product formation at 232 nm. A unit of enzyme activity was defined as an enzyme amount that catalyzes the formation of 1 μmol of product during 1 min. The amount of Δ-4,5-unsaturated pectin destruction products was calculated based on the molar extinction coefficient $\varepsilon_{235} = 5500\ M^{-1}\cdot cm^{-1}$.

Another method used in this work for determination of pectin lyase activity was based on the ability of the enzymes to decrease the viscosity of pectin solutions [8]. A unit of viscosimetric activity was defined as the enzyme amount corresponding to a 50% decrease in relative viscosity of the solution of citrus pectin (MD 70%) during 5 min at pH 5.0 and 40°C.

Hydrolytic activity towards PGA was determined by the initial rate of formation of reducing sugars (RS) using the Somogyi–Nelson method [9].

Isolation and purification of pectin lyase. Isolation of pectin lyase from *P. canescens* preparation consisted of the three steps: preliminary purification, anion-exchange chromatography, and hydrophobic chromatography. The preliminary purification step included precipitation with ammonium sulfate (80% saturated, 25°C), centrifugation at 4°C for 30 min (8603*g*), resuspension of the pellet in 0.1 M sodium acetate buffer, pH 5.0, and desalting on a column with Acrylex P-2 (Reanal, Hungary) using an Econo-System liquid chromatograph (Bio-Rad, USA) and 0.02 M Bis-Tris-HCl, pH 6.0, as an eluent at a flow rate of 1 ml/min. The two next purification steps were performed by liquid chromatography using a Pharmacia FPLC system (Sweden). Anion-exchange chromatography used a Source 15Q column (Pharmacia); unbound protein was eluted with 0.02 M Bis-Tris-HCl buffer, pH 6.0, at a flow rate of 5 ml/min. Hydrophobic chromatography was performed on a column with Source 15Q Isopropyl carrier (Pharmacia) equilibrated with 1.7 M $(NH_4)_2SO_4$ in 0.05 M sodium acetate buffer, pH 5.0. Bound protein was eluted by the buffer with linearly decreasing concentration of ammonium sulfate at a flow rate of 2 ml/min.

Protein content in the samples was determined by the Lowry method [10] using BSA as a standard or by absorbance at 280 nm.

Determination of biochemical characteristics of pectin lyase. Analytical isoelectrofocusing of proteins was carried out using a Model 111 Cell instrument (Bio-Rad) according to the manufacturer's instructions. Protein electrophoresis under denaturing conditions (in the presence of SDS) was performed in 12% polyacrylamide gel using a Mini Protean system (Bio-Rad). Protein bands were stained with Coomassie Brilliant Blue R-250 (Ferak, Germany). Protein mixtures MW-SDS-200 (30-200 kD) and IEF-M1A (p*I* 3.6-9.3) (Sigma) were used as standards for SDS-PAGE and isoelectrofocusing, respectively.

Isolation and sequencing of pectin lyase gene. Isolation of the phage clone with *P. canescens* pectin lyase gene and DNA sequencing was performed according to a previously described method [11].

Search for homologous proteins and construction of a three-dimensional model of pectin lyase. The search for homologous proteins was performed using the BLAST2 software (http://cn.expasy.org/tools/) (http://afmb.cnrs-mrs.fr/CAZY). Three-dimensional structure of *P. canescens* pectin lyase was obtained by the homologous modeling method using a SWISS-MODEL software from the Swiss Bioinformatics Institute (http://swissmodel.expasy.org/SWISS-MODEL.html) [12] and known structures of pectin lyases A (PL A) and B (PL B) (EC 4.2.2.10) from *A. niger* as templates.

Determination of temperature and pH optima for pectin lyase activity. The following buffers were used in the study of pH dependence of pectin lyase activity: Polybuffer 96 (Pharmacia)/H_2O/CH_3COOH (pH 7.0-9.2) and Polybuffer 74 (Pharmacia)/H_2O/HCl (pH 2.6-7.4).

Determination of kinetic parameters for hydrolysis of specific substrates. Kinetic parameters (K_m, V_{max}) for the reaction of pectin hydrolysis by pectin lyases were determined from the dependences of initial rates of trans-elimination reaction (registered by kinetics of accumulation of unsaturated products at 232 nm) on substrate concentration plotted using Lineweaver–Burk analysis.

Complete hydrolysis of pectins. The enzyme was incubated with pectin solution (5 g/liter) at pH 5.0 (0.1 M

sodium acetate) at 25°C for 24 h. Aliquots of the reaction mixture were taken during the hydrolysis reaction, and the concentration of Δ-4,5-unsaturated products of the *trans*-elimination reaction was determined by absorbance at 232 nm.

Study of pectin lyase stability. In the study of pectin lyase stability, the enzyme solution (600 μl) was incubated for 3 h at 40°C and pH 4-6. The following buffers were used for adjustment of the pH of the medium: 0.1 M sodium phthalate (pH 4.0), 0.1 M sodium acetate (pH 5.0), and 0.2 M sodium maleate (pH 6.0). Aliquots of the solutions were taken after equal periods of time (15-60 min), and the activity towards citrus pectin with MD of 70% was measured.

During the study of pectin lyase activity under thermal shock conditions (imitating the processes of pasteurization at 70°C and enzyme granulation at 80°C), the time intervals between collecting the aliquots were 15-60 sec.

Effect of calcium and sodium ions and EDTA on pectin lyase activity. A solution of citrus pectin with MD of 70% was preliminarily incubated with an effector (the following concentrations were used: 0-0.1 M for $CaCl_2$, 0-1 M for NaCl, and 1 mM for EDTA) for 10 min at 40°C and pH 5.0 (0.05 M sodium acetate). Pectin lyase activity was then determined using a standard technique.

Production of cranberry juice using pectin lyase. Cranberries were carefully washed, dried in air, and homogenized. To 50 g of the berry homogenate, 0.5 ml of enzyme preparation was added (in a control experiment 0.5 ml of distilled water was added). Dosage of enzyme preparations was performed by taking equal protein content. Berry homogenate was treated for 1.5 h at 40°C and natural pH value. In the course of the experiment a volume of the juice was filtered through a paper filter during 15 min, and relative viscosity of the juice was calculated as a ratio of filtering time for the juice and distilled water, determined using an Ostwald's viscosimeter.

Estimation of pectin lyase efficiency in clarifying apple juices. Unclarified apple juice (5 ml) was incubated in a thermostatted shaker at 45°C for 10 min. Then 0.1 ml of enzyme preparation was added, mixed thoroughly, and the reaction mixture was incubated during 150 min at the natural pH (3.5). Aliquots of 1 ml were taken after 100 and 150 min, mixed with 2 ml of 90% isopropanol [13], and the formation of high molecular weight pectin precipitate was monitored (precipitation time 3-5 min). In the course of the experiment, such a minimal enzyme dose was chosen that there was no precipitate formation observed after 150 min of juice treatment.

RESULTS AND DISCUSSION

Isolation of homogeneous pectin lyase from the enzyme complex secreted by *P. canescens*. The enzyme preparations from *P. canescens* PCA-10 were purified from non-protein admixtures (insoluble substances, carbohydrates, pigments, etc.) and applied to a Source 15Q anion-exchange chromatography. Pectin lyase activity was detected in the unbound fraction eluted before the salt gradient. According to SDS-PAGE data, this fraction contained a considerable amount of other proteins; therefore, it was subjected to subsequent separation by hydrophobic chromatography on a Source 15 Isopropyl column. Pectin lyase activity was detected in the third major fraction eluted in a salt gradient (Fig. 1). This fraction was additionally purified by gel filtration on Sephadex G-25. As a result, homogeneous pectin lyase (as determined from SDS-PAGE and isoelectrofocusing data) was obtained with a molecular weight of 38 kD and p*I* 6.7. It should be noted that the molecular weight of the majority of known pectin lyases is 33-49 kD [14].

Isolation and sequencing of *P. canescens* pectin lyase gene. Homogeneous pectin lyase was subjected to SDS-PAGE. The gel region containing the protein band was excised, treated with trypsin, and a MALDI-TOF mass spectrum of the trypsinized hydrolyzate was obtained (these experiments were performed at the Department of Proteomic Studies of the Institute of Biomedical Chemistry, Russian Academy of Medical Sciences). Then the method of tandem (TOF/TOF) mass-spectrometry [15] was used to determine amino acid sequences of three peptides with the highest intensities in MALDI-TOF spectrum. Fungal pectin lyases were found in the protein database SWISS-PROT using BLAST2 software (http://an.expasy.org/tools/), whose amino acid sequences were homologous to the isolated peptides. Two peptides were selected, the corresponding nucleotide sequences were determined, and PCR primers synthesized. A fragment of genomic DNA encoding the gene of

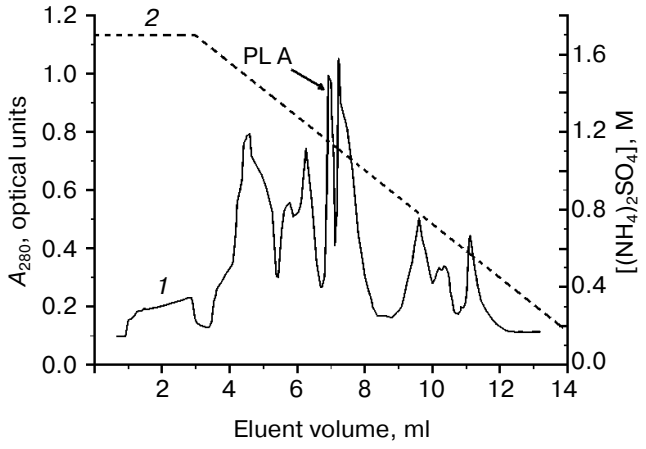

Fig. 1. Isolation of PL A from *P. canescens* PCA-10 enzyme complex: hydrophobic chromatography on a Source 15 Isopropyl carrier. The unbound fraction after anion-exchange chromatography of the initial preparation on a Source 15Q column is shown. *1*) Protein, A_{280}; *2*) $(NH_4)_2SO_4$ concentration gradient.

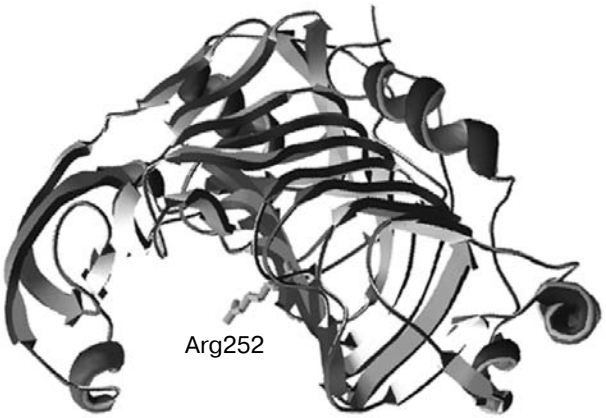

Fig. 2. Model of three-dimensional structure of *P. canescens* PL A. α-Helices are shown as cylinders and β-sheets as arrow lines. The location of a potential catalytic residue Arg252 is shown schematically.

pectin lyase was isolated from *P. canescens* gene library cloned in a phage vector [11]. The full nucleotide sequence of the gene encoding pectin lyase (*pelA*) was determined, and the amino acid sequence of the protein containing 360 residues was predicted based on the nucleotide sequence. The theoretical molecular weight of the protein was 37,546 daltons, which virtually coincides with the molecular weight of the purified protein, determined from SDS-PAGE (theoretical p*I* value is 5.95). There were no possible N-glycosylation sites found in the amino acid sequence of the *P. canescens* pectin lyase.

Search for homologous proteins and construction of three-dimensional model of *P. canescens* pectin lyase. The search using the BLAST2 software demonstrated that the amino acid sequence of *P. canescens* pectin lyase is similar to that of the first family of polysaccharide lyases (all pectin lyases known today belong to this family [14]). The greatest similarity of the primary structure (67%) was found in the case of *A. niger* pectin lyase A [16] (Swiss-Prot accession code Q01172). Moreover, the *P. canescens* enzyme exhibited similarity of amino acid sequence with *A. oryzae* pectin lyase I (Q8X1X2, 66%) [17], *A. niger* pectin lyase B (Q00205, 63%) [18], *A. oryzae* pectin lyase II (Q8X1X1, 62%) [19], *A. niger* pectin lyase D (PL D) (P22864, 58%) [20], and two pectin lyases from *P. griseoroseum* (Q8NKE3 and Q8NKE4, 52 and 51%). The *P. canescens* enzyme isolated by us was classified as pectin lyase A (gene *pelA*).

As reference structures for comparative modeling of three-dimensional structure of *P. canescens* PL A, we used the known three-dimensional structures of crystalline PL A and B from *A. niger* (PDB codes 1IDJ and 1QCX) [21, 22]. The resulting three-dimensional model is schematically represented in Fig. 2. The protein globule has a β-helix shape in the right arm conformation. The inner side of the active site groove is a substrate-binding region and contains a large number of aromatic (Trp, Tyr, Phe) and nonpolar (Val, Leu, Ile, Ala) residues responsible for binding of highly methylated pectin (not shown in the picture).

A potential catalytic residue (Arg252) acting as a base and being highly conservative in pectin and pectate lyases has been identified in the *P. canescens* PL A molecule by comparative analysis of the aligned protein amino acid sequences and superimposition of the three-dimensional model with known structures of PL A and B from *A. niger*. The fragment of the aligned sequences of homologous fungal pectin lyases containing this residue is shown in Fig. 3.

Substrate specificity of *P. canescens* PL A. Among pectins with different degree of substitution, *P. canescens* PL A preferably hydrolyzed highly etherized substrate with a MD of 89%; high activity of PL A towards this pectin was observed both during the detection of specific lyase reaction by formation of Δ-4,5-unsaturated products at 232 nm and using viscosimetric detection (Table 1). Using less substituted substrates (MD 70 and 65%) the enzyme activity decreased, and in regards to the last pectin in this series (with the lowest MD value of 26%) the enzyme was virtually one order of magnitude less active than compared to the first substrate. PL A has practically no effect on completely demethylated substrate (PGA). The results (preferable hydrolysis of highly methylated pectins) are a typical feature of most pectin lyases described in the literature [6, 14, 23, 24].

Determination of kinetic parameters for hydrolysis of specific substrates. The values of kinetic parameters of PL A catalyzing the cleavage of pectins with different MD are given in Table 2. One should note the decrease in K_m value

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PEL A  A. niger          198  HYVLGTSADN RVSLTNNYID GVSDYSATCD GYHYWGIYLD GDADLVTMKG NYIYHTSGRS PKVQDNTLLH CVNNYFYDIS
Pel I  A. oryzae         199  HIVLGTQADN RVTISNSLID GRTDYSATCN GHHYWGVYLD GSNDMVTMMG NYFYYTSGRM PKVQGNTLLH AVNNYFHNIE
PL  B  A. niger          197  HIVLGTSADN RVTISYSLID GRSDYSATCN GHHYWGVYLD GSNDMVTLKG NYFYNLSGRM PKVQGNTLLH AVNNLFHNFD
Pel II A. oryzae         197  HYVLGTEADN RVTLSNNYID GESDYSATCD GHHYWNVYLD GSSDKVTMKG NYFYKTSGRA PKVQGNTYLH AVNNYWNDNS
PL  D  A. niger          197  HYVLGTDADS RVSITNNYIN GESDYSATCD GHHYWNVYLD GSSDKVTFSG NYLYKTSGRA PKVQDNTYLH IYNNYWENNS
Pel F  A. niger          200  MFVAGYEASH SVTISNSEFD GETSWSATCD GHHYWTVLGY GHNDKITFAN NYIHHTSGRS PKLEFNSFWH AYNNYWYNNT
PL  A  P. canescens      194  HIVLGTEASK RVTISNSFIN GASDYSATCD GYHYWGIYLD GSNDYVTMKG NYIYHTSGRA PKVQGSTLLH AVNNYWYDNS
Plg 2  P. griseoroseum   203  ALRPGTEADN RVSITNNYIN GESDYSATCD GHHYWNVYLD GSSDKVTFKG NYLYKTSGRA PKVQDNTYLH AVNNYWDENS
                                * **    **   *  *  *  ******  *****  *** *  * **  * ** * ****  **** ** **  ***
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Fig. 3. Fragment of *P. canescens* PL A sequence aligned with sequences of homologous pectin lyases of fungal origin. Highly conservative regions are underlined; other conservative residues are marked with an asterisk; the residue responsible for catalysis is selected in a frame.

Table 1. Specific activities of *P. canescens* PL A (40°C)

Substrate	Source	pH	Additives	Activity, unit/mg	Comments
Pectin, MD 70%	citrus	5.0	EDTA no	28.0 27.2	pectin lyase activity
		8.0	EDTA no	2.2 2.1	
PGA	orange	5.0	$CaCl_2$ no	0.007 0.006	pectate lyase activity
		8.0	$CaCl_2$ no	0.004 0.003	
«	«	5.0	«	0.26	polygalacturonase activity
Pectin, MD 26%	citrus	5.0	«	6.62	pectin lyase activity
Pectin, MD 65%	«	5.0	«	26.5	
Pectin, MD 89%	«	5.0	«	48.2	
Pectin	orange apple beat	5.0 5.0 5.0	« « «	340-380 380 0.66	viscosimetric activity

and increase in k_{cat} value with the increase in MD of pectin from 26 to 89%. These results are in agreement with the conclusions about higher specificity of pectin lyases towards highly methylated pectin drawn by other researchers [14, 25].

Complete pectin hydrolysis by pectin lyase. Extensive hydrolysis of pectins with different MD has been studied. PL A cleaved most efficiently highly methylated pectin: the degree of substrate conversion for pectins with MD of 0 (PGA), 26, 65, and 89% upon complete destruction was 0, 2, 10, and 16%, respectively. The addition of a fresh portion of enzyme did not increase the concentration of unsaturated products. This indicated that the termination of reaction was not due to enzyme inactivation.

Effect of calcium ions on PL A activity. Pectin lyases do not require the presence of calcium ions, but the latter still can have an effect on enzyme activity [14, 26, 27]. Thus, at pH values above the optimum, calcium ions can increase pectin lyase activity or even shift the pH optimum into more acidic range. To date there is no general theory for the effect of cations on pectin lyase activity, but most researchers assume that the major effect of bivalent cations is directed towards the substrate, changing its conformation, total charge, and aggregation degree. Sensitivity of some pectin lyases to the presence of sodium ions in solution [26] can be explained in a similar way.

No effect on enzyme activity toward highly methylated citrus pectin by *P. canescens* PL A was displayed by NaCl and $CaCl_2$ at the salt concentration of 0-1 and 0-0.1 M at pH 5.0 and 8.0, respectively (Table 1). Moreover, the presence of chelating agent (EDTA) in the reaction mixture had no effect on catalytic properties of PL A (Table 1).

Dependence of PL A activity on pH and temperature. The dependences of PL A activity on pH and temperature are shown in Fig. 4. PL A exhibited maximal activity in acidic medium at pH 5.0-5.5. The optimal temperature was 60°C. It should be noted that less acidic pH range is typical for the majority of fungal pectin lyases [14, 28]; for instance, the pH optimum for *A. niger* PL B is 8.5 [24]. The optimal temperatures for known pectin lyases are 45-60°C [6, 28].

PL A stability. An important feature of pectin lyase in terms of its practical application is stability. The ability of

Table 2. Kinetic parameters for hydrolysis reaction of pectins with different etherification degrees catalyzed by *P. canescens* PL A (40°C, pH 5.0)

MD, %	K_m, g/liter	k_{cat}, sec^{-1}
26	2.9 ± 0.4	25 ± 1
65	2.5 ± 0.3	90 ± 5
70	1.7 ± 0.2	95 ± 7
89	1.2 ± 0.1	133 ± 10

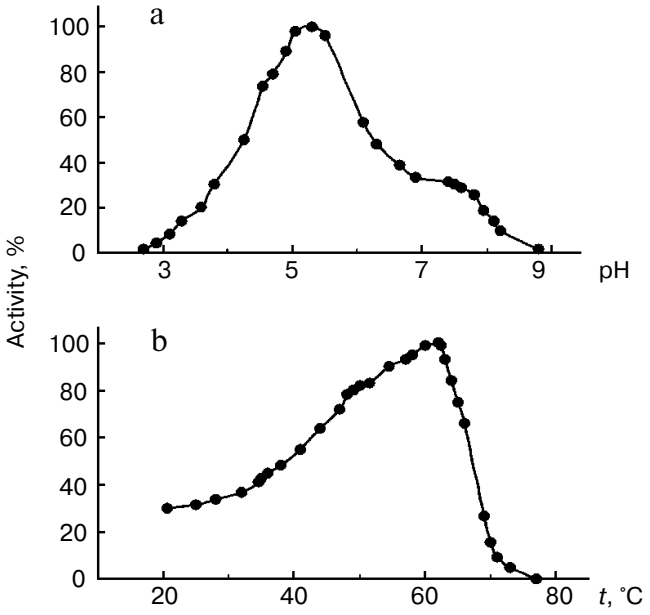

Fig. 4. Dependences of *P. canescens* PL A activity on pH at 40°C (a) and on temperature at pH 5.0 (b) determined by *trans*-elimination reaction of highly methylated citrus pectin.

PL A to maintain activity during prolonged exposure to increased temperatures (40-60°C) was studied at pH 4-6. The highest stability was displayed by the enzyme at pH 5.0-6.0, where the residual activity after 3 h incubation at 40 and 50°C was 80 and 60%, respectively. Shift of the pH into more acidic range (pH 4.0) slightly decreased PL A stability: the enzyme retained 70 and 40% of its initial activity after 3-h incubation at 40 and 50°C, respectively. Upon the increase of temperature to 60°C the enzyme activity decreased more dramatically: PL A retained only 20% of its initial activity after 1-h incubation at pH 5.0, whereas at pH 4.0 and 6.0 the activity decreased to zero after 30-40 min.

Enzyme solutions had low stability to short-term exposure to high temperatures: half-inactivation time for PL A at 70°C (pasteurization temperature) was 120, 150, and 180 sec in solutions with pH 6.0, 4.0, and 5.0, respectively. The dry form of an enzyme was characterized by a considerably higher stability at high temperatures. Stability of the dry enzyme form was studied at 80°C (granulation temperature) at different humidity values. Thus, at a humidity of 2-3%, PL A preserved 95% of its initial activity after incubation during 7 min; at a humidity of 15%, enzyme half-inactivation time was 120 sec.

Therefore, on one hand, *P. canescens* PL A has sufficient stability under granulation conditions (80°C, dry enzyme form), and on the other hand, it was easily inactivated in solutions at 70°C, i.e. under conditions of juice pasteurization.

Estimation of PL A efficiency for production of fruit juices. The efficiency of potential PL A application for juice manufacturing was estimated using production of cranberry juice and clarification of apple juice by way of example. Two known commercial pectinase preparations (Rapidase Press and Rapidase C-80L) from the fungus *A. niger* were chosen as internal standards. The first preparation recommended by the manufacturer for production of juice from berries contained polygalacturonases, pectin esterase, pectin lyase, as well as a number of hemicellulases. The second preparation, specifically produced for juice clarification, had high polygalactouronase and pectin esterase activities.

Production of juice from berry raw materials (especially in the case of wild berries with high pectin content) without enzymatic treatment is complicated: the pulp has low drainage ability, thus impeding juice output. The use of enzymes increases the juice output and dramatically reduces its viscosity [29]. The results of experiments on the application of PL A for production of juice from berry raw materials (using cranberry by way of example) are shown in Fig. 5. One should note the dramatic advantage of PL A during the enzymatic treatment compared to control (the absence of enzymes): the yield of drained (free-run juice) in the control sample was 5 ml (at the relative juice viscosity of 9 units), whereas treatment with *P. canescens* PL A increased the yield of cranberry juice up to 16 ml (at the same time the relative juice viscosity

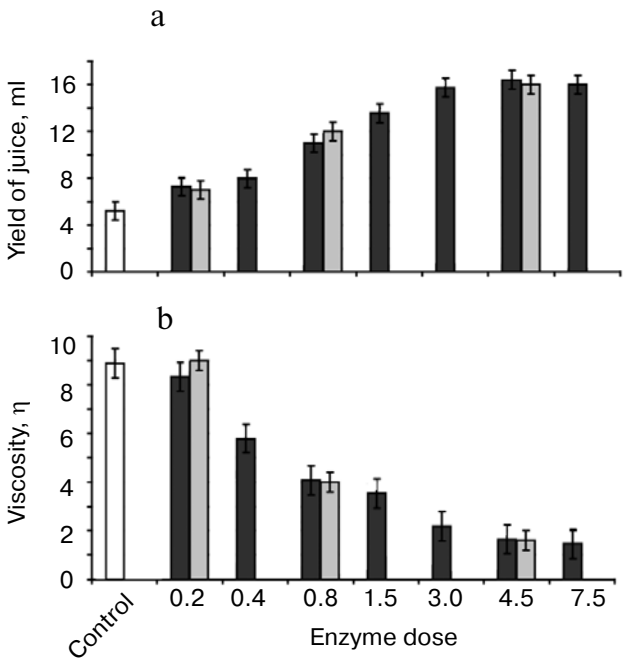

Fig. 5. Results of *P. canescens* PL A trials using cranberry juice production by way of example (black bars). a) Yield of cranberry juice; b) viscosity of cranberry juice. The data obtained for a commercial preparation of Rapidase Press (gray bars) and a control experiment in the absence of enzyme (white bars), are shown for comparison. Enzyme dose is expressed in grams of protein per ton of berries.

decreased to 1.3 units). Based on the data in Fig. 5, PL A dose of 0.4-0.8 g protein per ton of raw materials can be recommended for treatment of wild berries such as cranberry. For comparison, Fig. 5 also shows the results of an identical experiment using a commercial preparation of Rapidase Press taken at a dosage recommended by the manufacturer (70-100 ml per ton of berry raw materials).

We have also studied the ability of *P. canescens* PL A to clarify (depectinize) commercially available non-clarified apple juice. The criterion for efficiency was the minimal possible dose of the enzyme (enzyme preparation) completely degrading high molecular weight juice polysaccharides during the 2.5 h, which resulted in the absence of high molecular weight polysaccharide pellet upon precipitation with ethanol. In the course of experiments performed, it tuned out that the efficient treatment of 1 liter of juice requires 0.2 mg of homogeneous PL A. The minimal possible dose of commercial Rapidase C-80L preparation, determined by us experimentally, was 7 μl, which is also equivalent to 0.2 mg protein. It should be noted that the dose of Rapidase C-80L recommended by the manufacturer is 15-20 μl per liter of juice.

Therefore, we have isolated and characterized the new pectin lyase A secreted by the mycelial fungus *P. canescens*, and the corresponding *pelA* gene has been isolated and sequenced. High efficiency of PL A for production and clarification of juices has been demonstrated.

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