EXPERIMENTAL ARTICLES

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METALLOPROTEASE FROM THE CULTURAL LIQUID OF *Pleurotus ostreatus*

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The aim of this work was to identify and to study physical and chemical properties of the enzyme preparation which was obtained from the cultural liquid of *Pleurotus ostreatus*.

The protease containing fraction was obtained from the cultural liquid by sodium chloride precipitation followed by dialysis and concentration procedures. Gelatinase and milk-clotting activity were defined by standard methods. The content of the protein component of the fraction was analysed by HPLC, Laemmli electrophoresis and MALDI-TOF analysis. Protease activity was proved by enzyme-electrophoresis. To identify the protease, mass-spectrometry was carried out using the MatrixScience database. To study the specificity of protease action, the series of chromogenic substrates were used: S2238; S236; S2251; S2765; Leu-pNa; Ala-pNa and S2302. The inhibitory analysis was carried out using EDTA, benzamidine, PMSF, PCMB.

The obtained fraction possessed maximal protease activity at 45 °C. Meanwhile maximal milk-clotting activity was observed at 35 °C. The highest milk-clotting activity was shown at pH 5.0 and less than 3.0. The highest protease activity was shown at pH 6.0. Using HPLC method, it was found the main protein component and some minor proteins. According to the electrophoresis results, the main protein component of the fraction had molecular mass 45 kDa. Enzyme electrophoresis demonstrated that protease activity of the fraction was present in the zone corresponding to 45 kDa. When identifying trypsinolysis products, no homology was found with other known proteinases. It was shown that the protease hydrolyzed peptide bonds which were formed by carboxyl group of amino acids with hydrophobic side chains. The enzyme was inhibited by EDTA (IC50 = 2.5 mM). The maximal enzyme activity towards gelatin and Leu-pNa was shown in the presence of 5 mM calcium chloride.

The new calcium-dependent metalloprotease with molecular weight 45 kDa was found in the cultural liquid of *P. ostreatus*. The enzyme had no homology with other known proteases and hydrolyzes peptide bonds formed by carboxyl groups of amino acids with hydrophobic side chains.

Key words: basidiomycetes, proteolytic enzymes, milk-clotting activity, physical and chemical properties.

Proteases of animal origin became common use in dairy industry in particular in cheese production. Nowadays, the main sources of these proteases are pancreas and gastric mucosa of cattle and pigs. This resource is limited, so substitution of the expensive rennet enzyme by mushroom proteases is cost effecting and promising. It was shown that the level of milk-clotting activity of basidiomycetes was comparable to that of the traditionally used commercial rennet enzymes [1].

The requirements for rennet substitutes are strict. Their enzymatic properties should be as close as possible to natural renin. This means that, along with high milk-clotting activity, substitute enzymes should have a slight total proteolytic activity [1, 2]. Due to the concomitant proteolytic activity,

the obtained clots often have a bitter taste, which negatively affects the quality of cheese products [1, 3]. An analysis of literary sources shows that the search for substitute enzymes in the macromycete group is quite successful [4, 5]. It is known that *Pleurotus ostreatus* contains proteases with milk-clotting activity (MCA) [3, 6]. There are data that the extract of fruit bodies of *P. ostreatus* is similar to preparations used in the dairy industry and after purification can be used in cheese making.

In our previous investigations we selected nutrient media and optimal conditions for the deep cultivation of *P. ostreatus* [7]. We showed that the enzyme preparation from the cultural liquid of *P. ostreatus* possessed both: milk-clotting and proteolytic activity [8, 9].

Materials and Methods

Obtaining of the protease containing fraction. The experiments were performed on a "wild" strain of *P. ostreatus*, which was isolated from fruiting bodies growing on a cultivated poplar (*Populus* sp.). In all experiments, a potato-sucrose medium was used, as described in [7]. The mycelium was planted under a laminar box to minimize the risk of contamination. The inoculum was introduced in the form of fragments of a carpet of stock culture of mycelium with an area of 1 cm^2. Cultivation was carried out for 14 days in dark at a temperature of 27 °C on a shaker of WiseShake SHO model at 70 rpm. At the end of the incubation, the culture liquid was collected and frozen. As the initial stage of purification of the enzyme preparation from the culture liquid, salting out with sodium chloride (100% saturation) was used.

The salt was removed by dialysis. For long-term storage of the preparation, the method of freeze drying was used with a combination of a temperature of −51 °C and a pressure of 1.370 mBar. Protein concentration was determined spectrophotometrically [10].

Gelatinase activity. Proteolytic activity (PA) was determined according to the method described by Leighton et al. [11]. A mixture containing 0.15 ml of the enzyme preparation and 0.25 ml of the substrate (1% gelatin in 0.2 M acetate buffer, pH 5.0) was incubated for 60 min in the absence of light (gelatin final concentration 6.85×10^{-6} M). The reaction was stopped by the addition of 10% trichloroacetic acid. The mixture was centrifuged (8.000 rpm) for 15 min at 4 °C. Then 1.4 ml of 1 M NaOH was added to the obtained supernatant (0.8 ml). For one unit of PA, an enzyme amount was taken that promotes an increase in absorbance of 0.01 in one hour at 440 nm.

Milk-clotting activity. Milk-clotting activity (MCA) was determined according to the Pyatnitsky method: a test tube with a substrate (milk 3.4% volume 10 ml) containing a 0.0015 M solution of calcium chloride was heated to 35 °C and 2 ml of the studied enzyme preparation was added. The preparation activity was evaluated by the time of formation of a dense milk clot. The unit of MCA was the amount of enzyme that clots 100 ml of milk in 40 min at 35 °C [12].

To identify the optimum pH of the enzyme preparation, PA and MCA were determined at 25 °C with gelatin in various pH ranges using the following buffer solutions: 0.2 M acetate (pH from 3.8 to 5.8) and 1M phosphate (pH from 5.8 to 8.0).

To determine the temperature optimum, the enzyme preparation was incubated with gelatin at temperature in the range from 25 to 80 °C. When studying the effect of preincubation on MCA and PA, the enzyme preparation was preincubated at various temperatures in the range from 25 to 80 °C for 1 hour. After that, the determination of proteolytic activity was carried out as described above.

The effect of calcium ions on the milk-clotting activity of the enzyme preparation was determined by adding a solution of calcium chloride to a substrate (milk) in a final concentration from 20 to 500 mM. The samples were incubated at 60 °C, after that milk clotting activity was determined.

The effect of calcium ions on the protease activity was studied as it was explained above for milk except the gelatin that was used as the substrate of the reaction (see 'Gelatinase activity' section).

Electrophoretic analysis was performed in 12 and 10% PAAG by the Laemmli SDS PAGE [13]. Protein zones were identified after Coomassi R-250 staining.

Enzyme electrophoresis was performed to identify protein zones with fibrinogenase activity. Gel was polymerized in the presence of 0.5 mg/ml fibrinogen. After electrophoresis performed by the above method, DS-Na was removed from gel by three times washing in 2.5% solution of Triton X-100. The gel was then incubated in 0.1 M glycine buffer, pH 8.3 for 12 h. The gel was stained with Coomassi R-250 and the areas of proteolytic activity identified by the location of the unstained spots on the gel.

HPLC on phenyl sepharose. Chromatographic system Agilent 1100 was used for the extract analysis with column Dupont Instrument (250 mm long and 4.7 mm over) with ZorbaxSilicogel(20 μm) with phenyl inoculation in pressure of 140 bar and flow 1.5 ml per minute. Two buffer gradients were used: the decreasing one (0.15M TrisHCl pH 6.5, 0.13M NaCl) and increasing one (50% acetonitrile contained buffer with 0.1% trifluor acetic acid).

MALDI-TOF analysis of trypsinolysis products of the main protein component from the cultural liquid of *P. ostreatus* was performed using a Voyager-DE (Applied Biosystems, USA). H+-matrix ionization of polypeptides under sinapic acid (Sigma-Aldrich) was used. The results were analyzed by Data Explorer 4.0.0.0 (Applied Biosystems) [14].

Amidase activity was determined by cleavage of chromogenic substrates: S2238 (H-D-Phe-Pip-Arg-pNa), S236 (pyro-Glu-Pro-L-Arg-pNa), S2251 (D-Val-Leu-Lys-pNa), S2765 (Z-D-Arg-Gly-Arg-pNa), Leu-pNa, Ala-pNa, S2302 (H-D-Pro-Phe-Arg-pNa). The assay was performed in microplates, which wells were successively introduced with 0.05 M Tris-HCl buffer pH 7.4 and a chromogenic substrate in the final concentration 20 μM. The reaction was started by adding an enzyme-containing fraction at 37 °C. Amidase activity was characterized by the rate of release of paranitroaniline (pNa), which was detected at a wavelength of 405 nm using a Multiskan EX reader [15].

Protein concentration was determined according to Bradford [16].

Statistical analysis was performed using STATISTICA 6.0 software ($n = 5$).

Results and Discussion

Total protease and milk-clotting activity

The influence of pH

Milk-clotting activity (MCA) of the enzyme preparation was observed in a narrow pH range from 3.6 to 5.6. The pH optimum of the enzyme preparation with MCA was represented by two peaks at pH 3.6 and pH 5.0. The proteolytic activity of the enzyme preparation from *P. ostreatus* was observed in the entire pH range from 3.6 to 8.0. The pH optimum of proteolytic activity was at pH 7.0 (Fig. 1).

According to the literature, the proteases of some fungi are active in a wide pH range. For proteases derived from *P. ostreatus* fruiting bodies, the pH range, at which proteolytic activity is maintained, was in the range from 4 to 9 [3]. The stability interval of milk-clotting proteases from *P. ostreatus* mycelium was in the pH range from 3.5 to 7.5 [4]. These data are consistent with our results regarding the effect of pH on the activity of proteases from *P. ostreatus* culture liquid. It was shown that with high proteolytic activity of the enzyme preparation, not only the formation of a clot was observed, but also its further hydrolysis. This leads to the appearance of bitter peptides and makes such an enzyme preparation unsuitable for use in the cheese production. Taking into account these data, we recommend for making cheese the use of the enzyme preparation with a pH value of 3.6, since at this pH the ratio of MCA/PA is 74: 1. For example, at pH 5 the ratio of MCA/PA is only 13: 1 (Table 1).

The influence of temperature

In order to investigate the physicochemical properties of milk-clotting proteases from

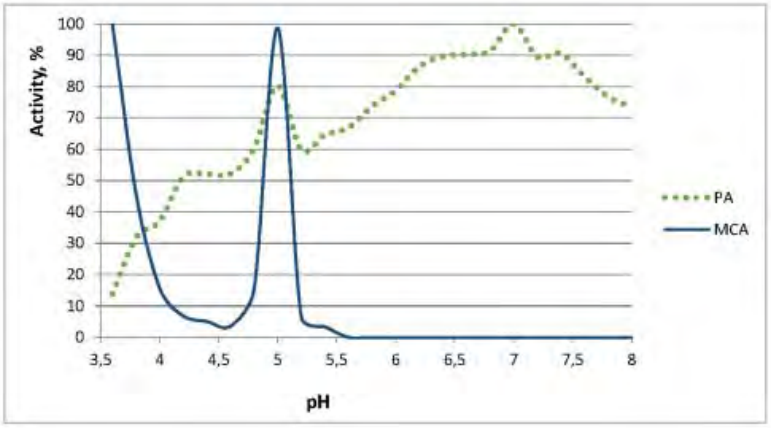

Fig. 1. **The influence of pH on the protease activity (PA) and milk-clotting activity (MCA) of the enzyme preparation from the cultural liquid of *P. ostreatus***

The milk-clotting and protease activity of enzyme preparation at different pH values

pH	Protein mg/ml	Total MCA (U*)	Specific MCA (U/mg of protein)	Total PA (U)	Specific PA (U/mg of protein)	MCA/PA
3.6	1.07	81.08	75.78	1.09	1.02	74:1
3.8	1.07	37.27	34.8	2.5	2.34	16:1
4.0	1.07	12.74	11.9	2.92	2.73	4:1
4.2	1.07	5.57	5.2	4.03	3.77	1.5:1
4.4	1.07	4.12	3.92	4.01	3.75	1.1:1
4.6	1.07	2.89	2.7	4.11	3.8	0.8:1
4.8	1.07	12.35	11.5	4.74	4.43	2.6:1
5.0	1.07	80	74.77	6.32	5.91	13:1
5.2	1.07	5.38	5.03	4.71	4.4	1.2:1
5.4	1.07	2.72	2.54	5.09	4.76	0.6:1

* U-milk-clotting or protease activity unit.

P. ostreatus culture liquid, we studied the effect of temperature on the milk-clotting and proteolytic activity of the enzyme preparation. To study thermostability, we conducted a series of experiments in which the preparations were preincubatedat under various temperatures for 1 hour. The temperature optima for PA and MCA were different.

The maximum milk-clotting activity was observed at 45 °C (Fig. 2). This value coincides with that for *Pleurotus eringii* and is somewhat lower than for the fungi enzymes and *Tricholoma saponaceum* (55 °C) [17, 18]. The milk-clotting activity is maintained up to 55 °C at pH 5. A further temperature increase sharply inactivates the milk-clotting enzymes of the studied fungus. At 4 °C, the milk-clotting activity of the lyophilic powder solution remains at the same level for a month. The obtained data are consistent with the results obtained for the enzyme preparation from the fruit bodies of *P. ostreatus* [4, 5].

The proteolytic activity of the enzyme preparation from *P. ostreatus* was observed in the entire temperature range from 25 to 60 °C (Fig. 2). The temperature optimum of proteolytic activity is at 45 °C. This value is comparable with the temperature optimum, which was previously determined for the enzyme preparation from *P. eringii* [19].

As it can be seen from Fig. 2 and 3, during one hour of pre-incubation of solutions containing enzymes at 35 and 45 °C, an increase in MCA and PA was observed, at least 2 times, respectively. This phenomenon was discovered earlier by other researchers for an enzyme preparation containing MCA from the fruit bodies of *P. ostreatus* [4].

Theinfluence of calcium ions on MCA

There was no influence of calcium ions on protease activity of the enzyme preparation. The concentration range was from 2 till 50 mM. However, the influence of calcium ions on the milk-clotting activity was significant. There is evidence in the literature that calcium stimulates the activity of milk-clotting enzymes. Calcium taken at a sufficiently high concentration was considered as an important component in the formation of the milk clot [18]. The addition of 1.8 μM calcium chloride to milk improved its coagulation and led to an increase in milk clot hardness by 32% [19]. The cheese hardness could be increased to 81% due to addition of 10 mM $CaCl_2$. However, an increase in calcium chloride concentration caused a decrease in cheese hardness [19, 20]. The use of high concentrations of calcium chloride could have negative effects on cheese production.

The use of high concentrations of calcium chloride changed the meltability of the cheese clot that caused a number of problems in cheese production [20]. As can be seen from Fig. 4, the maximum milk-clotting activity in our studies was achieved when calcium chloride was added to the substrate (milk) at a final concentration of 10 mM.

Our data differ from those for the enzyme preparation of microbial origin. Thus, the milk-clotting activity of proteases from *Bacillus amyloliquefaciens* was highest at

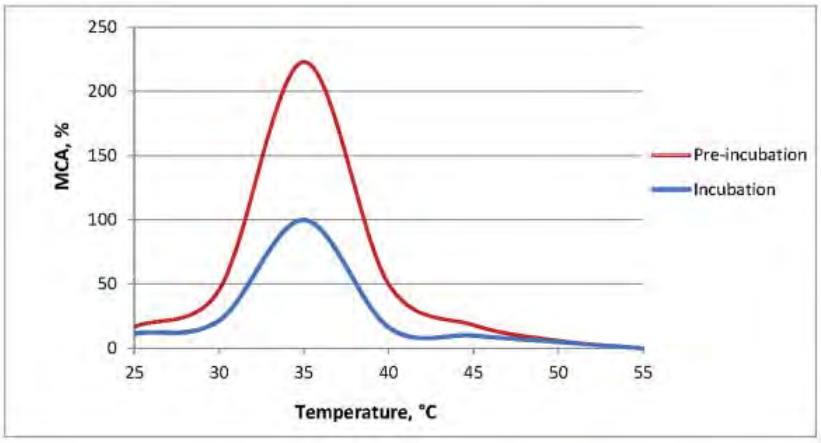

Fig. 2. **The influence of temperature on milk-clotting activity (MCA) of the enzyme preparation from the cultural liquid of *P. ostreatus***

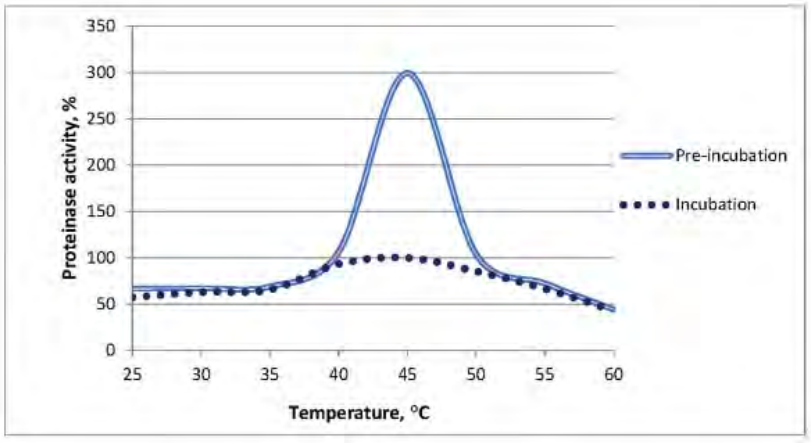

Fig. 3. **The influence of temperature on protease activity (PA) of the enzyme preparation from the cultural liquid of *P. ostreatus***

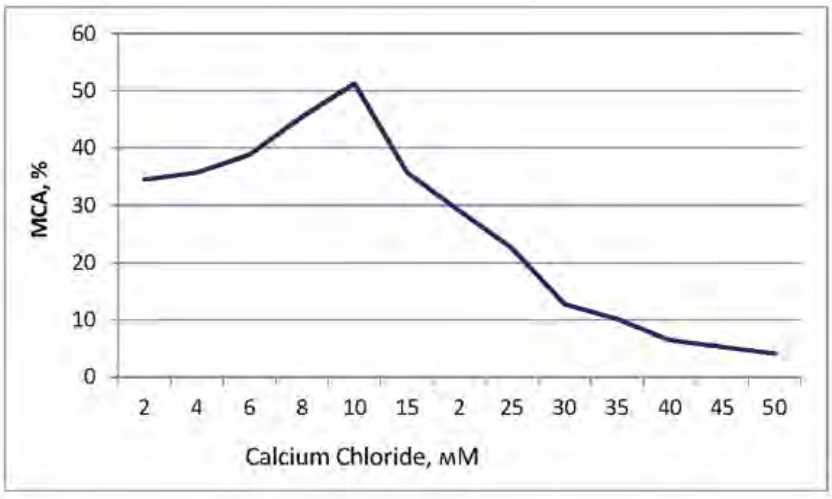

Fig. 4. **The influence of calcium chlorideon MCA of the enzyme preparation from cultural liquid of *P. ostreatus***

a final concentration of calcium chloride of 25 mM. In the range of calcium chloride concentrations from 0 to 20 mM, the milk-clotting rate increased with an increase in Ca^{2+} ions concentration. Meanwhile at a concentration above 25 mM, a decrease in milk-clotting activity was observed [21].

Maximum MCA of the protease from *Enterococcus faecalis* was obtained by adding 50 mM calcium chloride to the incubation medium [14]. The effect of CaCl2 on the aggregation of para-casein micelles is explained by its effect on the average coagulation rate. It was hypothesized that electrostatic repulsions and ionic bonds played an important role in the interaction between chymosin and para-casein [22, 23].

Identification of the protease component from the cultural liquid of P. ostreatus

According to electrophoresis results, main protein component of the fraction had molecular mass 45 kDa (Fig. 5). Using HPLC method it was found the main protein component and some minor proteins (Fig. 6). So, HPLC data proved the electrophoresis results and gave us possibility to concentrate our efforts on identification of the major component of the fraction. To prove the enzyme activity of the protein component, enzyme electrophoresis was applied with fibrinogen as a standard substrate. It was shown that protease activity of the fraction was present in the zone corresponding 45 kDa (Fig. 7).

To identify the enzyme, trypsinolysis of the main protein component was carried out followed by the analysis of its products using MALDI (Fig. 8). Identification of the trypsinolysis products of the main protein component let us to carry out the screening of its amino acid sequence and make the comparison of this sequence with other sequences of known enzymes from different origins. There was no homology with other known proteases.

Thus, protein with molecular weight of 45 kDa had proteolytic activity in the composition of *P. ostreatus* liquid culture. This enzyme, according to the results of the MALDI-TOF analysis of trypsinolysis products, did not present in publicly available databases and requires further investigation.

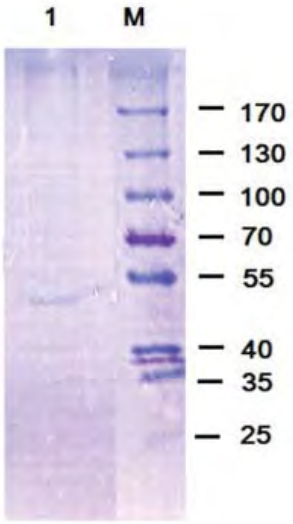

Fig. 5. **SDS-PAGE of the enzyme preparation from the cultural liquid of *P. ostreatus***

The study of hydrolytic activity of a protease from the cultural liquid of P. ostreatus

For a targeted investigation of the substrate specificity of proteases from *P. ostreatus* culture liquid, studies were carried out at pH of 7.4, to exclude the possible contribution of a milk-clotting enzyme. Amidolytic activity was evaluated using several chromogenic substrates: S2238 (H-D-Phe-Pip-Arg-pNa); S236 (pyro-Glu-Pro-L-Arg-pNa); S2251 (D-Val-Leu-Lys-pNa); S2765 (Z-D-Arg-Gly-Arg-pNa); Leu-pNa; Ala-pNa; S2302 (H-D-Pro-Phe-Arg-pNa).

As shown in Fig. 9, the enzyme has the highest specificity for Leu-pNa (among all investigated substrates) – the hydrolysis reaction rate was 0.22 μM/min. Previously, the highest amidase activity with the substrate S2586 (MeO-Suc-Arg-Pro-Tyr-pNA) was determined in the mycelium preparation of *P. ostreatus*. It is also known that chymosin (rennet) has specificity for peptide bonds formed by the C-group of hydrophobic amino acids. Chymosin specifically cleaves the Phe105-Met106 peptide bond in a casein molecule [22, 24–26]. It is known that the most specific substrate for chymosin is a compound with Phe-Met peptide bond. However we recommend Leu-pNa as more available substrate, which also has peptide lond formed by C-group of hydrophobic amino acid.

The effect of various inhibitors on amidolytic activity (with Leu-pNaas a substrate) is shown in Fig. 10. The enzyme from the cultural liquid of *P. ostreatus* was inhibited by 10 mM EDTA, a widely known inhibitor of metalloproteases (Fig. 10).

Moreover, the effect of EDTA had a concentration-dependent nature (Fig. 11). The determined IC50 value for EDTA was 2.5 mM. It

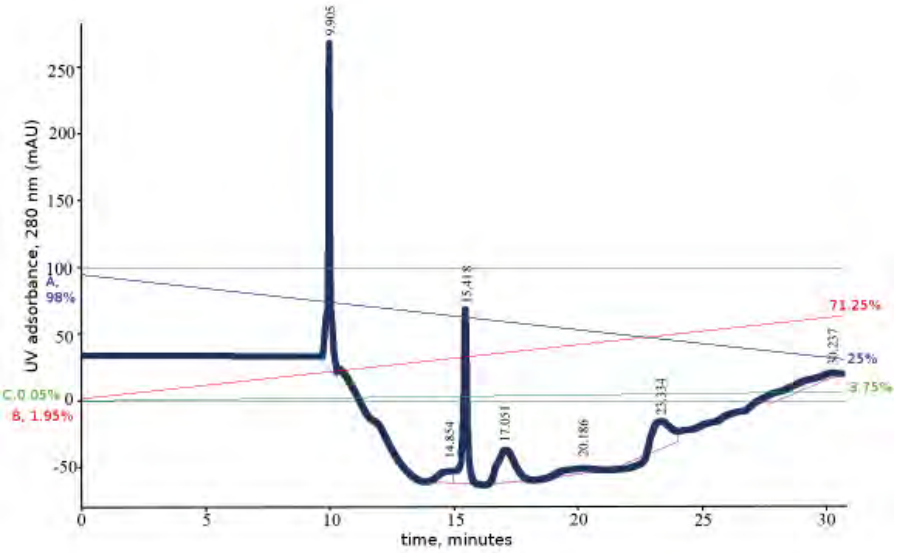

Fig. 6. **HPLC profile of the enzyme preparation from the cultural liquid of *P. ostreatus***

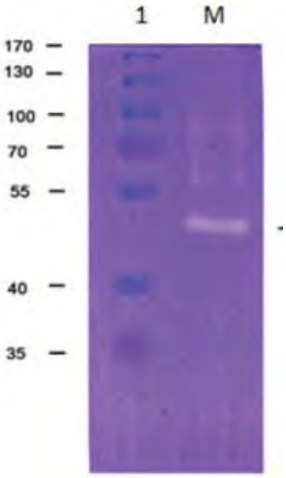

Fig. 7. **Enzyme-electrophoresis of the enzyme preparation from the cultural liquid of *P. ostreatus* using fibrinogen as a universal protease substrate**

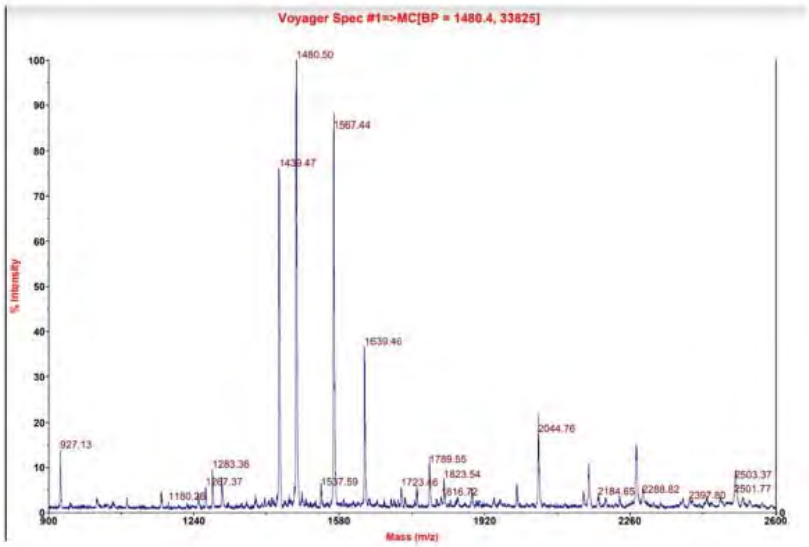

Fig. 8. **MALDI-TOF spectra of the trypsinolysis fragments of the main protein component from the cultural liquid of *P. ostreatus***

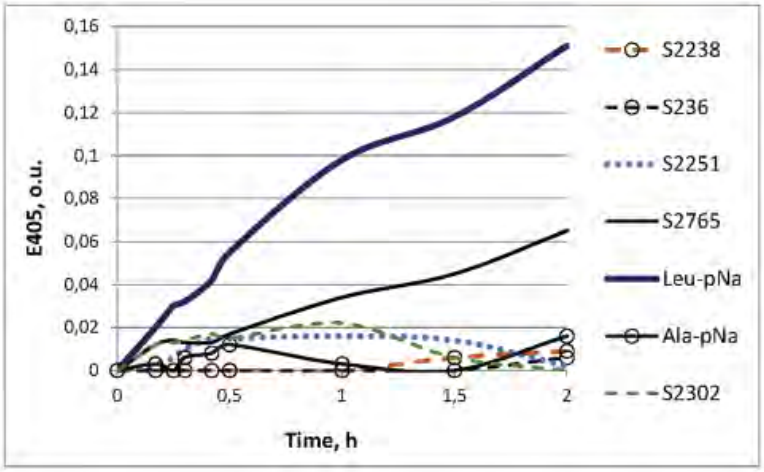

Fig. 9. **Amidase activity of the protease from the cultural liquid of *P.ostreatus* with the following substrates: S2238 (H-D-Phe-Pip-Arg-pNa); S236 (pyro-Glu-Pro-L-Arg-pNa); S2251 (D-Val-Leu-Lys-pNa); S2765 (Z-D-Arg-Gly-Arg-pNa); Leu-pNa, Ala-pNa; S2302 (H-D-Pro-Phe-Arg-pNa)**

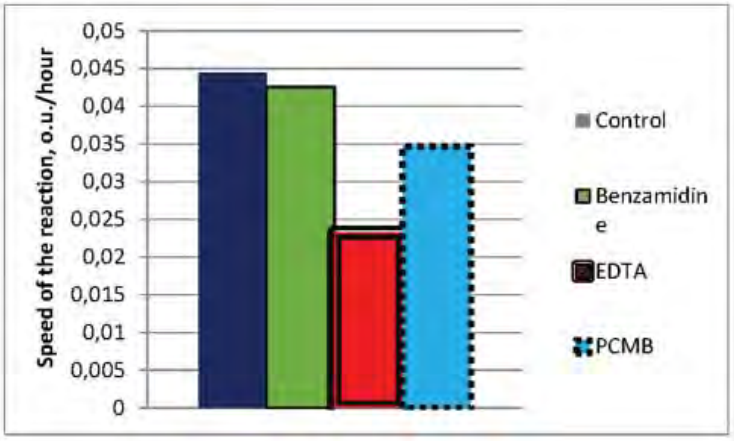

Fig. 10. **The rate of Leu-pNa degradation by protease from *P. ostreatus* cultural liquid in presence or absence (control) of the following inhibitors such as serine proteases (benzamidine), metalloproteases (EDTA), cysteine proteases (PCMB). Each inhibitor was taken at concentration 10 mM**

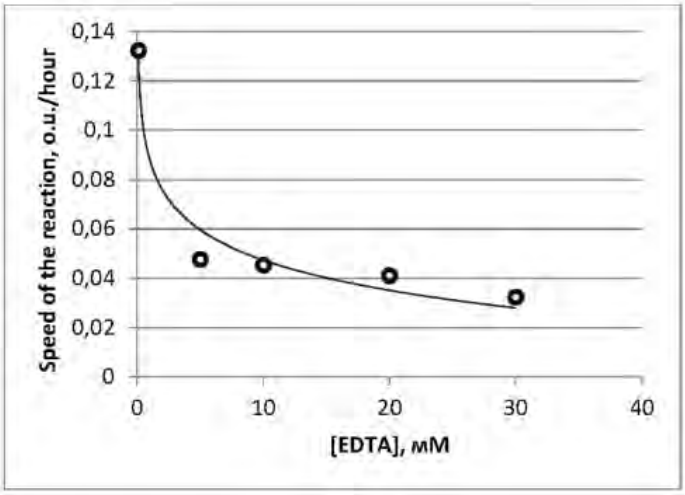

Fig. 11. **The effect of EDTA on the protease activity of the preparation from the cultural liquid of *P. ostreatus* with Leu-pNa as a substrate**

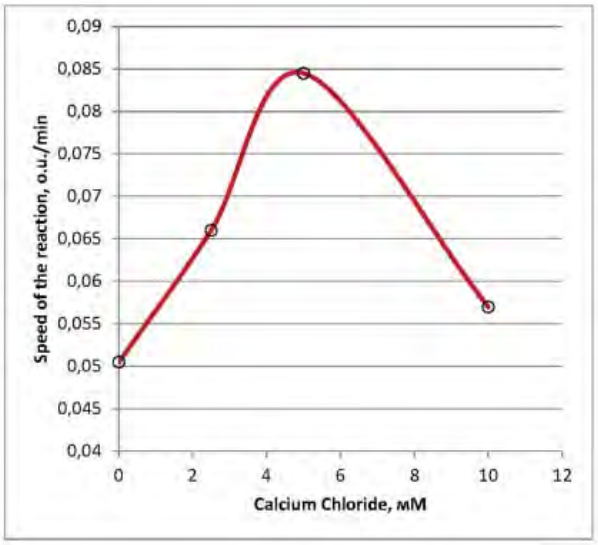

Fig. 12. **The effect of calcium chloride on the protease activity of the preparation from the cultural liquid of *P. ostreatus* with Leu-pNa as a substrate**

should be noted that the marked inhibitory effect of PCMB was not changed with the increasing of the inhibitor concentration and might indicate the presence of disulfide bonds in enzyme. Thus, the protease from the cultural liquid of *P. ostreatus* turns out to be a metalloprotease and, like many other proteases, is calcium dependent (Fig. 12). In particular, 5 mM calcium chloride activates the enzyme more than in 2 times.

Partially purified preparation from the cultural liquid of *P. ostreatus* contained a milk-clotting component, which was characterized for industrial application as a milk-clotting enzyme. According to our results, the recommended pH value was 3.6, optimal temperature was 35 °C. To increase milk-clotting activity we recommend one-hour preincubation of the enzyme preparation.

It was found the calcium-dependent metalloprotease with molecular mass 45 kDa in the cultural liquid of *Pleurotus ostreatus*. The enzyme had no homology with other known proteases and hydrolyzes peptide bonds formed by carboxyl groups of amino acids with hydrophobic side chains.

The work was conducted in the frame of the scholarship of Palladin Institute of biochemistry of the National Academy of Sciences of Ukraine given to Yevhenii Stohnii "Study of hydrolysis of fibrinogen molecule by proteases of different origin".

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МЕТАЛОПРОТЕЇНАЗА З КУЛЬТУРАЛЬНОЇ РІДИНИ *Pleurotus ostreatus*

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Метою роботи є виявлення і вивчення фізико-хімічних властивостей ензимного препарату, одержаного з культуральної рідини *Pleurotus ostreatus*.

Фракцію, що містить протеїназу, було отримано з культуральної рідини методом осадження хлоридом натрію з подальшим діалізом і концентруванням. Желатиназну і молокозгортальну активність визначали стандартними методами. Зміст протеїнового компонента фракції визначали за допомогою методів HPLC, електрофорезу за Леммлі та MALDI-TOF аналізу. Протеїназну активність вивчали ензим-електрофорезом. Для з'ясування специфічності дії протеїнази використовували низку хромогенних субстратів: S2238, S236, S2251, S2765, Leu-pNa, Ala-pNa і S2302. Інгібіторний аналіз проводили із застосуванням ЕДТА, бензамідином, ФМСФ, ПХМБ.

Отримана фракція виявляла максимальну протеїназну активність за 45 °С. Максимальну молокосідальну активність спостерігали при 35 °С. Найвищу молокозгортальну активність показано при pH 5,0 і менше 3,0. Найвища протеїназна активність була при pH 6,0. За допомогою методу HPLC було знайдено основний протеїновий компонент і деякі бічні протеїни. Згідно з результатами електрофорезу основний протеїновий компонент фракції мав молекулярну масу 45 кДа. Ензим-електрофорез проведено з використанням фібриногену як стандартного субстрату. Встановлено, що протеїназна активність фракції присутня в зоні, що відповідала масі 45 кДа. При ідентифікації продуктів трипсинолізу не виявлено гомології з іншими відомими протеїназами. Показано, що протеїназа гідролізує пептидні зв'язки, які утворені карбоксильною групою амінокислот з гідрофобними бічними ланцюгами. Ензим інгібували ЕДТА (IC_{50} = 2,5 мМ). Максимальну активність ензиму з желатином і Leu-pNa спостерігали в присутності 5 мМ хлориду кальцію.

У культуральній рідині *Pleurotus ostreatus* виявлено кальційзалежну металопротеїназу з молекулярною масою 45 кДа. Ензим не мав гомології з іншими відомими проїназами і гідролізував пептидні зв'язки, утворені карбоксильними групами амінокислот з гідрофобними бічними ланцюгами.

Ключові слова: базидіоміцети, протеолітичні ензими, молокозгортальна активність, фізико-хімічні властивості.

МЕТАЛЛОПРОТЕИНАЗА ИЗ КУЛЬТУРАЛЬНОЙ ЖИДКОСТИ *Pleurotus ostreatus*

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Цель работы — выявление и изучение физико-химических свойств энзимного препарата, полученного из культуральной жидкости *Pleurotus ostreatus*.

Фракция, содержащая протеиназу, была получена из культуральной жидкости методом осаждения хлоридом натрия с последующим диализом и концентрированием. Желатиназную и молокосвертывающую активность определяли стандартными методами. Состав протеинового компонента фракции определяли с помощью методов ВЭЖХ, электрофореза по Лэммли и MALDI-TOF анализа. Протеиназную активность изучали энзим-электрофорезом. Для выяснения специфичности действия протеиназы использовали ряд хромогенных субстратов: S2238, S236, S2251, S2765, Leu-pNa, Ala-pNa и S2302. Ингибиторный анализ проводили с использованием ЭДТА, бензамидина, ФМСФ, ПХМБ.

Полученная фракция обладала максимальной протеиназной активностью при 45 °С. При этом максимальная молокосвертывающая активность наблюдалась при 35 °С. Самая высокая молокосвертывающая активность была при pH 5,0 и менее 3,0. Самая высокая протеиназная активность была при pH 6,0. С помощью метода HPLC были найдены основной протеиновый компонент и некоторые побочные протеины. Согласно результатам электрофореза основной протеиновый компонент фракции имел молекулярную массу 45 кДа. Был проведен энзим-электрофорез с использованием фибриногена в качестве стандартного субстрата. Установлено, что протеиназная активность фракции присутствовала в зоне, соответствующей 45 кДа. При идентификации продуктов трипсинолиза не обнаружено гомологии с другими известными протеиназами. Показано, что протеиназа гидролизовала пептидные связи, образованные карбоксильной группой аминокислот с гидрофобными боковыми цепями. Энзим ингибировался ЭДТА (IC_{50} = 2,5 мМ). Максимальная активность энзима с желатином и Leu-pNa наблюдалась в присутствии 5 мМ хлорида кальция.

В культуральной жидкости *Pleurotus ostreatus* обнаружена кальцийзависимая металлопротеиназа с молекулярной массой 45 кДа. Энзим не имел гомологии с другими известными протеиназами и гидролизовал пептидные связи, образованные карбоксильными группами аминокислот с гидрофобными боковыми цепями.

Ключевые слова: базидиомицеты, протеолитические энзимы, молокосвертывающая активность, физико-химические свойства.

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