Study on Hemolytical and Antimicrobial Action of Poisonous Mushrooms Lectins of *Amanita virosa* Secr. and *Mycena pura* /Fr./ Kumm

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***Aim**.The aim of the present article was to study hemolytical and antimicrobical action of two new lectins obtained from the fruit bodies of poisonous basidial mushrooms A. virosa Secr. and M. pura /Fr./ Kumm. **Methods**. To study hemolytical action of lectins the human and animal erythrocytes were used. The experiments on osmotic protection of erythrocytes were performed in the presence of polyethylenglycols of different molecular mass (in a range from 400 to 4000 Da). Antimicrobical activity of lectins was studied by determination of growth delay area of cultures of different microorganisms on the Petri dish in agar medium. **Results.** Both lectins hemolyse the erythrocytes of rabbit, human, rat and dog and do not hemolyse the erythrocytes of cow and ship at concentration of 1 mg/ml. The rabbit erythrocytes are the most sensitive to hemolytical action of lectins, the hemolytic ability of lectin from A. virosa is the highest. Hemolysis was not observed in the presence of PEG with the molecular mass over 1,350 Da. Action of lectins on 10 types of microorganisms has been investigated. Basically, lectins inhibited growth of gram-positive microorganisms and Proteus (gram-negative microflora). For the most tested microorganisms, the antimicrobial action of Mycena lectin is stronger as compared with A.virosa lectin. **Conclusions**. Two new hemolytical lectins from the fruit bodies of mushrooms-basidiomycetes have been found. These lectins form ion-permeable pores in erythrocyte membranes with the hydrodynamic diameter smaller than 2.3 nm and larger than 1.6 nm. Above mentioned lectins also display antimicrobial activity and by the sum of these features are similar to the cytolytic lectins from lower invertebrates.*

Keywords: Amanita virosa, Mycena pura, lectins, hemolytical action, antimicrobical action.

Introduction. In our previous work, we studied carbohydrate specif icity of the lectin from *M. pure*/Fr./Kumm. mycena (abbreviation MPFA is derived from *Mycena pura* fungus agglutinin) and possibility of its application for histochemical investigations [1]. In that work, we determined that the above mentioned lectin weakly interacts with mono- and disaccharides, and much better interacts with glycoproteins that possess the highest affinity to alkaline phosphatase. However, as it was shown on histological slices of rat kidney and small guts of cow fetus, sensitivity of MPFA-binding receptors in mammalian tissues is not limited to alkaline phosphatase, but it probably spreads on other glyco-conjugates of complicated structure [1]. Despite high affinity to alkaline phoshatase, the *Mycena* lectin is able of

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hemolysing some species of erythrocytes. The last fact indicates that this lectin can be referred to a rare group of bifunctional hemolytical lectins, which contain, besides carbohydrate-binding domains, sites with cytolytical activity. Such lectins have been found in some invertebrates and only in two species of highest mushrooms, namely, *Laetiporus sulphureus* Bull. ex Fr. [2] *Amanita phalloides* (Fr.) Secr. [3], but not in plants. We have also revealed hemolytical lectins in related to *A. phalloides* species – *A. virosa* Secr. and *Mycena pura* /Fr./ Kumm. The latter is more distant species that belongs to *Tricholomataceae* genera.

This work is aimed to investigate the action of lectins, isolated from two poisonous basidial mushrooms *A. virosa Secr.* and *M. pura /Fr./ Kumm.*, on erythrocytes of mammals and on some pro- and eukaryotic microorganisms.

Materials and methods. *Determination of hemagglutinating activity and carbohydrate specifity of lectins.* Hemagglutinating activity of lectins was estimated, as described below. An identical volume of 2% suspension of erythrocytes in buffered saline solution (BSS) was added to the series of sequential dilutions of lectins in microtubes. After 10 min incubation at room temperature and centrifugation of microtubes at 500 g during 30s the result of agglutination was observed with a megascopic eye. Carbohydrate specifity of lectins was estimated according to the inhibition of hemagglutination by carbohydrates and glycoproteins. Minimal concentration of carbohydrate that totally inhibits lectin activity with titre of 1:4 was determined by a method of its stepwise dilution in microtubes. To characterize carbohydrate specifity of the lectin we used carbohydrates and glycoproteins that were previously referred and typified in details [1, 3, 4]. A detailed procedure of carbohydrate specifity determination was described earlier [9].

Evaluation of hemolytical activity was performed on erythrocytes of human, rat and rabbit as described in [3]. To carry out this procedure, the stock solution of purified lectin (1 mg/ml) was prepared. Then 0.05 ml of BSS was added to each of 10 microtubes. The stock solution of the investigated lectin was added to the first tube and then series of its two-fold dilutions were made. Therefore, lectin concentration in the 1-st tube was 0.5 mg/ml, in the second – 0.25 mg/ml, in the third – 0.125 mg/ml and so on. Next, 0.05 ml probes of 2% suspension of erythrocytes were added to each tube. We measured the least time of total erythrocyte hemolysis, which can be observed with an unaided eye in the corresponding tube. The graphic dependence of total hemolysis time on lectin concentration was constructed. The values of minimal concentration of lectins *A. virosa* and *M. pura* that were capable to induce hemolysis of erythrocytes of various animal and human origin are presented in Table 2.

Experiments on osmotic defence were performed in the presence of rabbit erythrocytes that appeared to be the most sensitive to lectin-induced hemolysis. The latest procedure was described in details previously [3]. Solutions of polyethylenglycol (PEG) of various molecular weight (PEG-400, 600, 1,350, 1,500, 3,000, and 4,000) were used in this experiment.

Study of antimicrobial action. Daily suspended cultures of *Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Klebsiella pneumoniae, Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris, Corynebacterium xerosis, Streptococcus faecalis* were sowed out as entire lawn in Petri dishes. Suspension of these microbes was prepared using the turbidity standard of 5 units. Each dish was divided into eight sectors, and 50 g of solutions of various lectins were dropped in the centre of each sector. Five trial series were carried out.

Besides the hemolytical lectins of mushrooms *A. virosa* Secr. were studied as well as *M. pura* /Fr./Kumm. lectins obtained from various sources, exhibiting distinct carbohydrate specifity (Scientific Manufacturing Complex "Lectinotest", Ukraine) such as the lectins from concanavalin A, *Helix pomatia*, *Sambucus nigra* bark, wheat germ, pea, Perca roe. To compare the inhibiting action we utilized discs with widely used antibiotics, e.g. gentamycin and nistatin. The cultures obtained were incubated in thermostat at 37 C during 24 h.

Results and discussion. The procedure of isolation of hemolytic lectins from the fruit bodies of *A. virosa* and *M. pura* mushrooms was described earlier [4]. Briefly, it includes the following stages.

The fruit bodies of mushrooms, collected in the Carpathian Mountains (Lviv region), were ground up in a mincing-machine, the obtained liquid was extruded

Table 1.
Minimal concentration of mono- and disaccharides inhibiting hemagglutination activity of lectins obtained from Amanita virosa and Mycena pura mushrooms.

Carbohydrate	Minimal concentration of carbohydrate required to inhibit activity of 4 Units of lectin, mM	
	M. pura lectin	*A. virosa* lectin
D-glucose	100	–
D-mannose	50	–
-methyl-D-mannopyranoside	25	–
Gentibiose (Glc 1,6Glc)	100	–
Turanose (Glc 1,3Fru)	100	–
Dipotassium salt of glucose-6-phosphateіль	100	–

N o t e. Both lectins do not interact with D-galactose, L-fucose, N-acetyl-D-galactopyranoside, -methyl-D-glucopyranoside, melibiose (DGal 1,6DGlc), maltose (DGlc 1,4DGlc), tregalose (DGlc 1,1DGlc), saccharose (DGlc 1,2DFru) in concentration of 100 mM, therefore, they are not included in the table.

and clarified by centrifugation, proteins were precipitated with ammonium sulphate (90 % of saturation) and the same purification procedure was performed in two cases (ion-exchange chromatography on DEAE-Toyopearl and affinity chromatography on the ovomucine [5]). 9 mg of purified lectins were yielded from 1 kg of the fresh fruit bodies of *M. pura*, and approximately 10 mg were obtained from the *A. virosa* fruit bodies.

Disc-electrophoresis in 10% PAAG in alkaline buffer system (pH 8.6) indicated that both lectin preparations had purity above 95 % [1, 4].

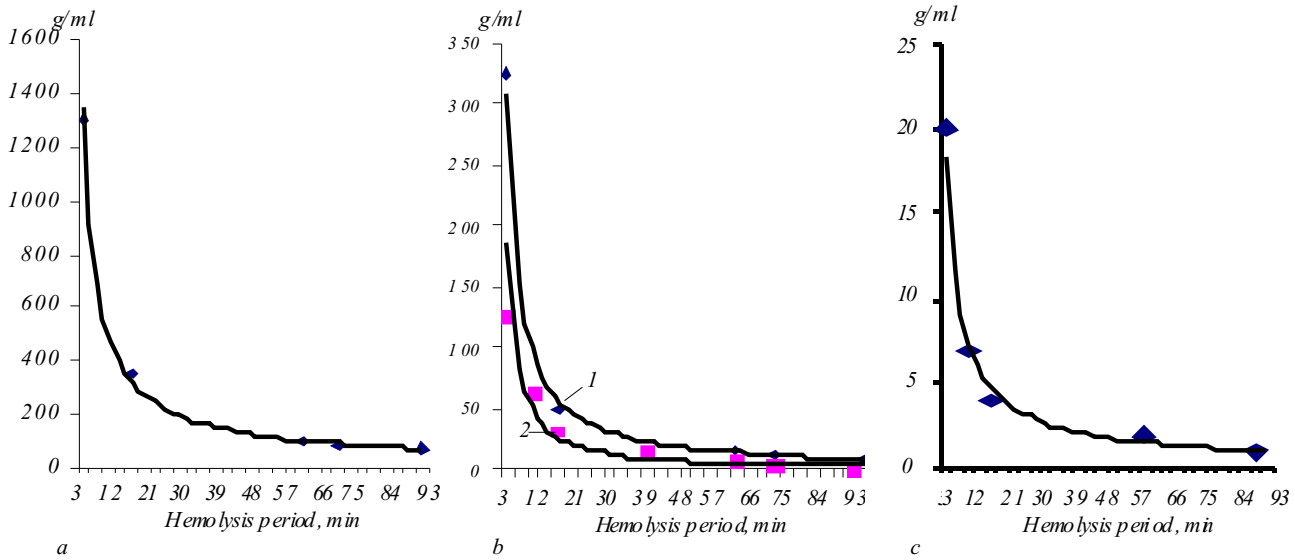
Lectins from the fruit bodies of *A. virosa* and *M. pura* differ from one another in the carbohydrate specificity. In particular, *M. pura* lectin interacts weakly with carbohydrates of D-mannose group (D-glucose, D-mannose, -methyl-D-mannopyranoside, gentibiose) (Table 1), whereas *A. virosa* lectin does not interact with monosaccharides. The] difference in lectin binding with some glycoproteins and polysaccharides is shown in Table 2. An analysis of the data presented shows that binding centres for carbohydrates in both lectins are much more complementary to oligosaccharide structures than to monosaccharide structures.

Table 2
Interaction of lectins of Mycena pura and Amanita virosa with polysaccharides and glycoproteins (the data from [1, 4] and present work)

Glycoprotein or polysaccharide	Minimal concentration of carbohydrate required to inhibit activity of 4 Units of lectin, mM	
	M. pura lectin	A. virosa lectin
Transferrin	0,25	0,25
Bovine thyroglobulin	0,125	0,125
Alkaline phosphatase of a calf intestine	0,002	0,125
Ovomucoid	0,06	0,125
Asialo-ovomucoid	0,015	0,25
Sheep submandibular mucin	0,125	0,03
Bovine submandibular mucin	1	0,25
Desialated bovine submandibular mucin	0,5	0,25
Human immunoglobulin G	–	0,08
$_2$-macroglobulin	1	0,03
Group-specific substance H, 1 %	0,125	0,03
Group-specific substance A, 1 %	0,125	0,03
Group-specific substance B, 1 %	0,25	0,06
Ovalbumin	0,125	–
Porcine liver glycogen 1 %	0,5	–
Yeast mannan, 1 %	–	–
Starch 1 %	1	–

N o t e. The through -line means the absence of interaction at lectin concentration of 1 %.

Lectin preparations obtained from *A. virosa* and *M. pura* hemolized erythrocytes of some animals and human. The results of research of lectin hemolitical and hem-agglutinating capacities are presented in Table 2.The data show that adding of the mycena lectin in concentration of 1 mg/ml to 2 % suspension of erythrocytes of sheep, cow and horse does not cause hemolysis. Alternatively, erythrocytes of dog, rabbit, human and rat are hemolysed with various intensity. The graphic dependence of erythrocyte hemolysis rate on the duration of action of lectins from *A. virosa* and *M. pura* is presented in the Fig.



The dependence of the rate of hemolysis of erythrocytes from various animal's species and human on duration of *A.virosa* lectin action: *a* – dog, *b* – human (*1*) and rat (*2*); *c* – rabbit (caption of Y axis is concentration that induces 100 % hemolysis). Graphs of the hemolysis rate dependence of the same erythrocytes on duration of *M. pura* lectin action are parallel with those of *A.virosa* ones and the only difference is numerical values, that is why they are not presented in the Figure.

Table 3.
Minimal concentration of lectins of Amanita virosa and Mycena pura inducing hemolysis and hemagglutination of erythrocytes of various animal's species and human

The source of erythrocytes	Minimal concentration, μg/ml			
	M. pura lectin		*A. virosa* lectin	
	Hemagglutination	Hemolysiз	Hemagglutination	Hemolysiз
Cow	125	> 1000	> 1300	> 1000
Sheep	16	> 1000	900	> 1000
Horse	8	> 1000	> 1300	600
Dog	8	250	40	60
Rat	2	12	600	1,5
Rabbit	0,06	4	0,02	0,1
Human, I (O)	4	30	80	7,5
Human, II (A)	1	20	80	3,8
Human, III (B)	2	30	40	7,5

The results presented indicate that rabbit erythrocytes are the most sensitive towards hemolytic action of the lectin, rat erythrocytes are less sensitive, while human, dog and horse erythrocytes are the least sensitive. As it was shown in Fig., there is a threshold concentration of lectin for each type of erythrocytes. It means that hemolysis does not occur at the lectin concentration lower than the threshold level. It comprises up to 600 μg/ml for horse erythrocytes, 60 μg/ml for dog, 3.8 – 7.5 μg/ml for human, about 1.5 μg/ml for rat erythrocytes, and up to 0.1 μg/ml for rabbit (see Table 3). The Mycena pure lectin hemolyses dog erythrocytes at approximately 4-fold higher concentration. Probably, this is the reason that lectin at concentration of 1 mg/ml does not hemolyse horse erythrocytes. However, rabbit erythrocytes, being the most sensitive to hemolysis action of both lectins, exhibit 40-fold difference between sensitivityвзаимно-с to *A. virosa* lectin and *M. pura* one. Thus, the hemolysis activity of these lectins can differ significantly for various erythrocytes.

Hemagglutinating and hemolysis action are mutually exclusive events. Observation of hemagglutination is possible only in the presence of high molecular weight substances, which do not inhibit it. PEG is considered to be the most appropriate substance of this type. It can be of different molecular weight, so there is a possibility to calculate the size of the molecules. The least PEG concentration that protects erythrocytes against hemolysis is 4 % for both lectins. Such concentration was the same for PEG-1,350, PEG-1,500,

Table 4
Diameter of zones of inhibited growth of various species of microorganisms under influence of the lectins of Amanita virosaand Mycena pura (50 μg of lectin per hole) and some

Microorganism	Diameter of delayed growth area, mm		
	M. pura lectin, 50 l	*A. virosa* lectin, 50 l	Gentamycin, 10 g
E. coli	–	–	24
P. aeruginosa	–	–	31
C. albicans	9	–	25 Nystatin, 80 Units
K. pneumoniae	20	14	7
S. epidermidis	25	18	35
S. aureus	28	17	26
B. subtilis	23	11	30
P. vulgaris	21	13	26
C. xerosis	33	22	36
Str. faecalis	22	24	32

N o t e. The through-line means absence of delayed growth area.

PEG-3,000 and PEG-4,000. Hemolysis was not observed in the presence of above-mentioned PEGs during 2 h incubation.

PEGs of molecular weight 600 and 400 Da do not protect erythrocytes against hemolysis induced by both lectins even at their concentration of 12 %. It indicates that the pores formed by the lectins in membranes of rabbit erythrocytes, are bigger than the hydrodynamic diameter of PEG-600, but less than that of PEG-1,350. According to [6], the hydrodynamic diameter of PEG-600 is 1.6 nm, while it is 2.3 nm for PEG-1,350. Therefore, both lectins form in erythrocyte membranes ion-permeable pores with diameter less than 2.3 nm and more than 1.6 nm. Previously we obtained similar results when the hemolytical lectin isolated from the fruit bodies of *A. phalloides* was studied [3].

Erythrocyte agglutination induced by both lectins, was observed in the presence of PEG-1,500 – PEG-4,000. The results of these experiments are presented in Table 3. There is no correlation between the hemolysis degree and erythrocyte agglutination. For instance, human erythrocytes are agglutinated at higher lectin concentration than dog erythrocytes, however, the latter are much more stable to the hemolysis caused by both lectins; rat erythrocytes are hemolysed by *A. virosa* lectin at lower concentration than human erythrocytes, but they display higher agglutinating activity.

It is supposed that both hemagglutinating and hemolytic activities of *A. virosa* and *M. pura* lectins are the functions of distinct (independent]) domains in the lectin molecule.

Studying lectin antimicrobical activity, scientists revealed that only hemolytic lectins from *Mycena pura* and *Amanita virosa* were capable of suppressing growth of microorganisms. The Mycena lectin mainly inhibits growth of gram-positive microorganisms as well as proteus (gram-negative microflora). In particular, the diameter of growth delay area for *C. xerosis* appeared to be 33 mm, *S. aureus* – 28 mm, *B. subtilis* – 23 mm. The lectin studied weakly influenced *C. albicans* and did not affect growth of *E. coli* and *P. aeruginosa*.

A. virosa lectin mainly inhibits growth of gram-positive microorganisms such as *C. xerosis, S. aureus, S. epidermides, K. pneumoniae, P. vulgaris, B. subtilis* , though it has no bacteriostatic effect on gram-negative microflora, namely *E. coli* and *P. aeruginosa*. The growth delay areas were found to be smaller than those for the mycena pure lectin (the case of *Str. faecalis* is an exception) (Table 4).

Antimicrobial activity of lectins was compared with the effects of known antibiotics, one of which, hentamycin, is an inhibitor of protein synthesis, and another one, nystatin, disrupts integrity of cell membrane. These data are also presented in Table 4.

Different action of hemolytic lectins of mushrooms towards gram-positive and gram-negative microorganisms can be explained by a diversity in their cell wall structure. Gram-positive bacteria, which are suppressed by lectins, have thick wall that does not contain lipids, whereas gram-negative microbe wall is thinner and contains large amount of glycolipids [7]. Hence, lectin action most likely depends on the presence of specific carbohydrates in cellular wall, but not on the wall thickness.

The Japanese group [8] investigated the effect of CEL-III-hemolytical Gal/GalNAc-specific lectin from *Cucumaria echinata* on artificial lipid membranes, which differed in the chemical composition. The formation of pores in the liposomes was observed in the

presence of lactose-containing glycolipids in membranes (but not glucose, with which CEL-III does not interact). The authors suggested that the leakage of internal contents of the liposomes depends on specific binding of CEL-III with carbohydrate chains on the liposome surface. Probably, similar mechanism occurs in the case of action of the above mentioned lectins towards microorganisms and erythrocytes from different species of animals.

Up to date, cytolytic lectins are discovered mainly in low vertebrates with primitive immune system. For these animals, recognition and lysis of foreign cells must be performed without antibodies of immunoglobulin nature, because they are absent there. In contrast to invertebrates, whose cytolysis is mediated by the complement system and includes a complex consisting of series of activated proteases, effector and regulator proteins, lysis of foreign cells, first of all microorganisms, in invertebrates is obviously achieved, in particular, with the participation of cytolytical lectins. The latter were not discovered in plants, and before the present work such lectins were described only in two species of fungi. Revealing similar lectins in two more species of high fungi points out a possible closeness of some groups of lectins from fungi to lectins from the low invertebrates. On the other hand, this fact may be an additional argument in favour of the opinion that the Sub-kingdom of Eumycota had separated from the general evolutionary mainstream somewhat later than the Sub-kingdom of plants.

Conclusions. 1. Two novel hemolytic lectins were revealed in the fruit bodies of basidial mushrooms, namely *Amanita virosa* Secr. (family *Amanitaceae*, genera *Agaricales*) and Mycena pura *M. pura* /Fr./Kumm. (family *Tricholomataceae*, genera *Tricholomatales*).

2. Both lectins in concentration of 1 mg/ml are capable of hemolysing erythrocytes of rabbit, human, rat and dog, but not of cow and sheep. Rabbit erythrocytes are found to be the most sensitive to hemolytical action of lectins. Minimal hemolysing concentration for *A. virosa* lectin was evaluated to be 0.1 g/ml, for *M. pura* – 4 g/ml. Hemolysing action of *A. virosa* lectin on tested erythrocytes is stronger than that of *M. pura*.

3. High-molecular substances with globular structure, which have hydrodynamic diameter of molecules over 2.3 nm (e.g. PEG-1,350), were capable to protect erythrocytes against hemolysis. It allowed us to study carbohydrate specificity and hemagglutinating activity of *A. virosa* and *M. pura* lectins by virtue of reaction of hemagglutinating suppression by carbohydrates and glycoproteins.

4. The action of lectins towards 10 microorganism species has been investigated. It has been found that *A. virosa* and *M. pura* lectins suppress predominantly the growth of gram-positive microbes and proteus. Antimicrobical activity of *M. pura* appeared to be stronger than that of *A. virosa* for the majority of the microorganisms tested.

5. The lectins from mushrooms-basidiomycetes *A. virosa* and *M. pura* are similar to the cytolytic lectins from lower invertebrates by the sum of properties studied (hemagglutination reaction, hemolysis and antimicrobial action).

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Дослідження гемолітичної та антимікробної дії лектинів отруйних грибів *Amanita virosa* Secr. та *Mycena pura* /Fr./ Kumm.

Резюме

Мета. *Дослідити гемолітичну та антимікробну дії двох нових лектинів, одержаних з плодових тіл отруйних грибів-базидіоміцетів A. virosa Secr. та M. pura /Fr./ Kumm.* ***Методи***. *Гемолітичну дію лектинів вивчали на еритроцитах людини і тварин. Експерименти з осмотичного захисту еритроцитів виконано за присутності поліетиленгліколю різної молекулярної маси (в діапазоні від 400 до 4000 Да). Антимікробну активність лектинів аналізували, визначаючи зону затримки росту культури різних видів мікроорганізмів на чашках Петрі в агаризованому середовищі.* ***Результати***. *Обидва лектини гемолізують еритроцити кроля, людини, щура та собаки і не гемолізують еритроцити корови й барана у концентрації 1 мг/мл. Найчутливішими до гемолітичної дії лектинів виявилися еритроцити кроля, гемолізуюча здатність лектину A. virosa є вищою. Гемолізу не спостерігалося за присутності поліетиленгліколю з молекулярною масою понад 1350 Да. Досліджено дію лектинів на 10 видах мікроорганізмів. Лектини пригнічують ріст переважно грампозитивних мікроорганізмів і протею. Для більшості випробуваних мікроорганізмів антимікробна дія лектину M. pura є силь- нішою, ніж лектину A. virosa Secr.* ***Висновки***. *Знайдено два нових гемолітичних лектини в плодових тілах грибів-базидіоміцетів. Вони формують у мембранах еритроцитів іоно-проникні пори, гідродинамічний діаметр яких є меншим за 2,3 нм, але більшим за 1,6 нм. Зазначені лектини виявляють також антимікробну активність і за сукупністю цих ознак нагадують цитолітичні лектини нижчих безхребетних.*

Ключові слова: Amanita virosa, Mycena pura, лектини, гемолітична дія, антимікробна дія.

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Исследование гемолитического и антимикробного действия лектинов ядовитых грибов *Amanita virosa* Secr. и *Mycena pura* /Fr./ Kumm.

Резюме

***Цель**. Исследовать гемолитическое и антимикробное действие двух новых лектинов, выделенных из плодовых тел ядовитых грибов-базидиомицетов A. virosa Secr. и M. pura /Fr./ Kumm. **Методы**.Гемолитическое действие лектинов исследовали на эритроцитах человека и животных. Эксперименты по осмотической защите эритроцитов выполнены в присутствии полиэтиленгликолей различной молекулярной массы (в диапазоне 400–4000 Да). Антимикробную активность лектинов анализировали, определяя зону задержки роста культуры разных видов микроорганизмов на чашках Петри в агаризованной среде. **Результаты**. Оба лектина гемолизируют эритроциты кролика, человека, крысы и собаки и не гемолизируют эритроциты коровы и барана в концентрации 1 мг/мл. Эритроциты кролика оказались самыми чувствительными к гемолитическому действию лектинов, при этом гемолизирующая способность лектина A. virosa выше. В присутствии полиэтиленгликоля с молекулярной массой выше 1350 Да гемолиз не наблюдался. Изучено действие лектинов на 10 видов микроорганизмов. Лектины преимущественно подавляют рост граммположительных микроорганизмов и протея. Для большинства исследованных микроорганизмов антимикробная активность лектина мицены выше, чем лектина A. virosa. **Выводы**. Найдены два новых гемолитических лектина в плодовых телах грибов-базидиомицетов. Они формируют ионо-проникающие поры, гидродинамический диаметр которых меньше 2,3 нм, но больше 1,6 нм. Указанные лектины обнаруживают также антимикробную активность и по совокупности этих признаков напоминают цитолитические лектины низших беспозвоночных.*

Ключевые слова: Amanita virosa, Mycena pura, лектины, гемолитическое действие, антимикробное действие.

REFERENCES

1. *Antonyuk V. O., Yastchenko A. M., Antonyuk R. V., Ambarova N. O.* Carbohydrate specificity of lectin, purified from the fruiting bodies of *Mycena pura* /Fr./ Kumm. (MRFA) and its uses in histochemical investigation // Biopolym. cell.–2009.–**25**, N 6.–P. 466–475.
2. *Tateno H., Goldstein I. J.* Molecular cloning, expression, and characterization of novel hemolytic lectins from the mushroom *Laetiporus sulphureus*, which show homology to bacterial toxins // J. Biol. Chem.–2003.–**278**, N 42.–P. 40455–40463.
3. *Antonyuk V. O.* Study of carbohydrate specificity of hemolytic lectin from death-cap mushroom (*Amanita phalloides* (Vaill. Fr.) Secr.) // Biopolym. cell.–2005.–**21**, N 4.– P. 1–7.
4. *Antonyuk V. A., Klyuchivskaya O. Yu., Stoika R. S.* Bifunctional hemolytic lectins from basidial mushrooms: studies hemolytic and toxic properties // Practical Phytotherapy (Moscow).–2009.–N 1.–P. 17–19.
5. *Pat*. (Author's sertificate) No 1554961. Method for obtaining of affinity sorbent for purification of lectin / V. A. Antonyuk // Bull. Izobret. USSR.–1990.–N 13. (In Russian).
6. *Scherrer R., Gerhardt P.* Molecular sieving by the *Bacillus megaterium* cell wall and protoplast // J. Bacteriol.–1971.–**107**, N 3.–P. 718–735.
7. *Kauzer F. H., Bienz K. A., Eskert J., Zinkernagel R. M*. Medical microbiology.–Stuttgart-New York: Thieme, 2005.–698 p.
8. *Kouriki-Nagamoto H., Hatakeyama T., Jelokhani-Niaraki M., Kondo M., Ehata T., Yamasaki N.* Molecular mechanism for pore-formation in lipid membranes by the hemolytic lectin CEL-III from marine invertebrate *Cucumaria echinata* // Biosci. Biotechnol. Biochem.–1999.–**63**, N 7.–P. 1279–1284.
9. *Lutsyk M. D., Panasjuk E. N., Antonyuk V. A., Lutsyk A. D., Ladnaya L. Ya*. Method for investigation carbohydrate specificity of lectins (Method. recommendation).–Lviv, 1983.–20 p.

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