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PROTEOLYTIC ENZYMES OF MYCELIAL FUNGI

The literature and the original experimental data on the synthesis of proteolytic enzymes by mycelial fungi, properties and application of proteases were analyzed. The wide distribution of the ability to produce proteases was demonstrated among fungi of various genera. Fungal proteases are distinguished by substrate specificity and broad range of optimal values for substrate hydrolysis, pH- and thermal stability. The multiple application aspects of fungal proteases and their catalytic characteristics determine the commercial prospects of fungi as the industrial enzyme producers.

K e y w o r d s : mycelial fungi-producers, proteolytic enzymes, properties, hydrolysis, application.

Proteolytic enzymes affiliated to hydrolases catalyze reaction of protein hydrolysis to peptides or free amino acids. Proteases are involved in the normal physiological processes of protein and cellular peptide biogenesis in the living species and in abnormal pathological processes. They can be applied in diverse industries, medicine, agriculture and are indispensable as research tools. Proteases originating from plants (papain, bromeline) and animals (trypsin, chemotrypsin, pepsin, rennin) are well-known and thoroughly examined [47]. Microorganisms are the most attractive sources of proteolytic enzymes, hardly limited by scale of production.

Proteolytic enzymes are synthesized by various microbial cultures — bacteria, yeasts, streptomycetes, fungi [7, 12, 20, 37, 40, 47]. Bacteria of genera *Bacillus*, *Lactobacillus*, *Fervidobacterium*, *Pseudomonas*, *Microbacterium*, *Yersinia* are recognized as producers of serine, cysteine and metal proteases, aminopeptidases [20, 47]. Bacteria of genus *Bacillus* are applied for industrial production of neutral and alkaline proteases. Representatives of genera *Candida* and *Trichophyton* synthesize aspartyl proteases, aminopeptidases, carboxypeptidases, dipeptidylpeptidases, *Streptomyces* — serine proteases [7, 37].

Mycelial fungi are the most promising cultures for large-scale manufacturing of proteolytic enzymes. They synthesize extracellular proteases belonging to various families and subfamilies, showing activity

and stability in wide pH spectrum, displaying enhanced substrate specificity and polyfunctionality.

The aim of this review is to sum up and analyze the literature and the original experimental findings on biosynthesis, properties and application of proteolytic enzymes from mycelial fungi.

Synthesis of proteolytic enzymes by mycelial fungi

The results of studies on the ability of fungi to synthesize proteolytic enzymes testify to wide distribution of this property among cultures of various genera belonging to different ecological groups. 2-stage screening of protease producers conducted at the Lab of enzymes, Institute of Microbiology, National Academy of Sciences of Belarus demonstrated that among 97 fungal strains representing genera *Aspergillus* (39 strains), *Penicillium* (56 strains) and *Paecilomices* (2 strains) 30% of tested cultures were able to liquefy gelatin, 40% — to yield clarified zones on agar media with defatted milk and 25% — on media with haemoglobin [53]. Seventeen fungal strains hydrolyzing at least 2 substrates were selected, and 11 of those synthesized extracellular proteases in submerged culture. The Indian researchers screened 221 isolate of deep-water fungi and sorted out 105 cultures (48%) distinguished by presence of protease activity [12]. Proteases were found out in fruit bodies of 18 species of basidial fungi, except *Polyporus* (*Coriolus versicolor* (Fr). Karst) and *Hydnum* (*Hericium epinaceus* (Fr.) Quel) [21]. Fungi of the families *Boletaceae* and *Agaricaceae* may be pointed out due to increased enzyme activity. The production of examined enzymes was revealed in nematode-, entomo-, phytopathogenic fungi and fungi pathogenic for humans and animals.

Mycelial fungi synthesize alkaline, acid and neutral proteases. Extracellular alkaline proteases are produced by *Aspergillus clavatus* [23, 52], *A. fumigatus* [60], *Penicillium chrysogenum* [10], *Paecilomyces lilacinus* F-2 [54], *Conidiobolus coronatus* [44], *Arthrobotrys olgospora* [57], *Fusarium culmorum* [43], *Trichoderma harzianum* [16], *Cephalosporium* sp. KM388 [55], etc.

Synthesis of acid proteases is typical for *Aspergillus niger* [27], *Sporotrichum pulverulentum* [17], *Penicillium griseoroseum* [24], *Trichoderma reesei* [22], *Trichoderma harzianum* [13], *Thermomyces lanuginosus* [32], etc.

Neutral proteases were detected in *Aspergillus carneus* [5], *A. sojae* [50], *Tricholoma columbetta* [29], *Fusarium culmorum* [60], etc.

Fungal proteases are the enzymes referred to diverse families and subfamilies. Pepsin-like aspartyl proteases were described in *T. reesei* [22], *T. harzianum* [13] and basidial fungi [21]. Trypsin-like protease was also produced by *Cordiceps militaris* [25]. Serine proteases were revealed in *T. lanuginosus* P_{134} [32] *and Conodiobolus* SP [51], while the enzyme of *Tricholoma columbetta* was classified as metal protease [29].

Fungi pathogenic for humans represented by 400 out of 10 000 described fungal species produce both endo- and exoproteases [37]. Aminopeptidases, carboxypeptidases and dipeptidylpeptidases were found in pathogenic fungi of genus *Aspergillus — A. fumigatus, A. flavus/oryzae*, *A. niger* [37]. *A. fumigatus* was also characterized by synthesis of aspartyl, serine and metal protease [28, 38, 48]. Proteolytic enzymes of dermatophytes were mainly described as keratinases and *Microsporum canis* was also shown to generate serine- [35] and metal proteases [9]. Proteases of pathogenic fungi are one of virulence factors and they play a key role in progress of certain pathologies.

Mycelial fungi are heterogenous eucaryotic heterotrophs and their structural-functional peculiarities determine lability of growth and metabolism depending on the components of nutrient media, the type and conditions of culture fermentation. The analysis of impact caused by carbon and nitrogen sources appears especially crucial for studies on protease biosynthesis by fungal strains. In nutrient media used for culturing producers of proteolytic enzymes the following substrates are used as C and N sources: sugars, biopolymers, mineral salts and organic compounds.

Maximal synthesis of *Thermomyces lanuginosus* P_{134} protease was reached in submerged culture on medium containing 4% casein, 4% glucose, 4% yeast extract [32]. *Conodiobolus* SP also produced extracellular protease during submerged fermentation on nutrient medium comprising glycerol, casein, peptone, soluble starch [51]. The application of Chapek medium with 1.0% casein ensured the synthesis of proteases in submerged culture by thermophilic fungi *Paecelomyces variotii* and *Aspergillus carneus,* isolated from the thermal springs of Bargusin valley [5].

The effect of nutrients, duration and temperature of fermentation (20—55 °C, 1—10 days), pH 4.0—8.0 on protease formation by *A. fumigatus* и *Penicillium sp*. was studied [42]. Medium composed of (g/l): KH_2PO_4 — 2.0; glucose — 1.0; peptone — 5.0; gelatin — 15.0 was used as basal. The maximal production of protease was achieved for *A. fumigatus* grown on glucose-peptone-gelatin medium for 4 days at pH 5.0, temperature 30 °C and for *Penicillium sp*. cultured on the same medium for 5 days at pH 6.0 and temperature 25 °C. Sucrose, ribose, raffinose stimulated protease generation in fungi.

The optimal composition of nutrient medium was established for synthesis of protease by *Botrytis cinerea* (g/l): KH_2PO_4 — 1.0; Mg (SO_4) — 0.3; molasses — 5.0; peptone — 5.0 ; yeast extract — 5.0; *Spirulina* algae — 2.0; KCl — 1.0; trace elements; pH 6.5 [1]. Fermentation was carried out for 9 days at 28 °C and agitation rate 150 rpm.

The comparative analysis of protease production by *Aspergillus clavatus* as a function of supplied sources of carbon (sucrose, glucose) and nitrogen (NH_4NO_3, $NaNO_3$, casein, gelatin) indicated that the peak level of enzyme synthesis was observed in 6-day submerged culture at 25 °C on media with

glucose and casein [52]. Starch (5 g/l) and yeast extract (2 g/l) proved the optimal carbon and nitrogen sources for strain *A. clavatus* ES1 [23].

The enzyme production is significantly influenced by the fermentation method. The solid-state technique of culturing fungal strains is frequently used to obtain proteolytic enzymes. Barley, rice, maize, soya, various food-processing wastes (fruit-berry pomace, bran, beet and potato pulp) mixed with the mineral salts and additives serve as appropriate substrates. Solid-state fermentation of microbial enzyme producers on media with above-mentioned substrates allows both to synthesize enzymes and to utilize vegetable wastes.

Screening of 30 *Penicillium griseoroseum* strains for protease production resulted in selection of the most active variant *P. griseoroseum* HHV-21 [24]. To optimize protease biosynthesis conditions effect of the following substrates on enzyme production there were tested: sunflower calathides, soya beans, rice hull, wheat bran. Soya cake proved the best carbon source.

During the solid-state culture of *Rhizopus oryzae* maximum enzyme yield (341 U/g wheat bran) was achieved at fermentation temperature 32 °C, pH 5.5, relative humidity — 90–95%, inoculation density — 2.10^5 spores/g wheat bran and 140% contents of solid substrate [56]. The medium containing wheat bran or wheat bran plus casein (humidity — 60%) was optimal for protease generation by thermophilic fungus *Thermoascus auranticus* [34].

The provided examples illustrate that mycelial fungi produce proteolytic enzymes in presence of protein substrates which may be evidence of the inducible type of enzyme synthesis.

In addition to nutrient sources and fermentation type the significant effect on fungal protease production is displayed by such factors as pH of the media and growth temperature.

The optimal conditions for protease biosynthesis are created in fungal culture on the media with initial pH lying in a broad range and at temperatures 28–30 °C [1, 19]. For instance, optimal growth temperature for production of alkaline protease in solid-state culture by fungus *Rhizopus oryzae* is 32 °C and pH 5.5 [56], whereas for *Penicillium griseoroseum* HUV-21 the optimal parameter is 30 °C [24]. The same temperature value and pH 6.0 are essential for formation of alkaline protease in *Aspergillus clavatus* ES 1 [23]. The other representatives of genus *Aspergillus* — *A. fumigatus* TKU003 and *A. fumigatus* Fresenius show the highest protease productivity when they are grown at temperatures 37 °C and 42 °C, respectively [49, 60].

Physical-chemical properties of proteases of mycelial fungi

Proteolytic enzymes synthesized by mycelial fungi differ in physical-chemical and catalytic properties defining application aspects of protease preparations.

The table presents physical-chemical characteristics of proteases from mycelial fungi of different genera. Acid proteases are the most active at pH 4.0—4.5, neutral proteases and metal proteases — at pH 7.0, while the best conditions for alkaline protease catalysis are created in pH range 7.0—11.0.

Proteases with similar and distinct properties have also been described in literature. For instance, *A. candidus* enzyme is able to hydrolyze casein at pH 11.0—11.5 and temperature 30 °C, whereas at 47 °C pH optimum is shifted to 7.0 [47]. Protease of *Trichoderma* spp. 5011 displays maximum activity at pH 6.5 and temperature 40 °C, stability in the range of pH values 3.0—10.0 and temperatures 30—80 °C [36]. The unique protease of *Fusarium culmorum* retaining activity in a broad temperature range 10—100 °C and pH zone from 4.5 to 8.5 was reported [60]. Maximal catalytic action was expressed by the enzyme at 50 °C and pH span 6.0—8.0.

The optimal conditions for serine protease of *Penicillium citrinum* are established at 45 °C and pH 6.5 [19]. The enzyme retains stability in pH range 6.0—9.0 and temperature span — 35—45 °C. It was found that ions of Co^{2+}, Mg^{2+}, Zn^{2+} inhibit activity of the enzyme, while ions of Ca^{2+} and Na^{+} — stimulate its activity. Protease displays stability in the presence of oxidants, like H_2O_2 and it is compatible with commercial detergents.

Maximum catalytic activity of *Trichoderma lanuginosus* P134 crude protease is expressed at temperature 70 °C and pH 5.0 and 9.0. The enzyme maintains stability in pH range 4.0—11.0 [32]. Protease retains 100% activity at 50 °C, and its half-life at 60 °C and 70 °C is 160 and 60 min, respectively.

Two acid proteases of fungus *Sporotrichum pulverulentum* causing wood white rot were purified and characterized [17]. It was demonstrated that molecular weight and isoelectric point of protease I (152-fold purification) constituted 28 kDa and 4.7, while similar parameters of protease II (127-fold purification) equaled 26 kDa and 4.2. The peak activity of the enzymes was recorded at pH 5.0 and 5.2, respectively. Both enzymes were inhibited by ions of Ag^{+}, Hg^{2+} and partially by Cu^{2+}.

Alkaline protease of *Aspergillus flavus* shows maximal catalytic activity at pH 7.5 and retains stability in pH span 8.0—11.0 [33] whereas protease of *A. tamarii* displays activity and stability in pH range 5.0—9.5 [8].

Protease of *Aspergillus clavatus* was purified by combination of ultrafiltration, alcohol precipitation and fractionation on DEAE cellulose and Sephadex-G 200. The enzyme showed maximal activity at 37 °C and pH 7.8 [41].

Extracellular protease of *Scytalidium thermophilium* hydrolyzes the substrate at pH 6.5—8.0 and temperature 37—45 °C [26]. The inhibitory analysis with *p*-chloromercurybenzoate, phenylmethyl sulphonyl fluoride, antipain, EDTA and pepstatin A led us to assume presence of thiol-containing serine protease.

Serine subtilysine-like protease of *Conidiobolus* SP was characterized by molecular weight (MW) 22 kDa, pI 8.2 and optimum pH 9.7 [51], while

Table

Properties of proteolytic enzymes of mycelial fungi

Microbial producer	Protease	MW, кDa	pI	Substrate used	Major ingibitor(s)	Optimum		Stability		Literature source
						pH	t, °C	pH	t,°C	
1	2	3	4	5	6	7	8	9	10	11
Aspergillus clavatus ES1	serine	32	ND*	casein	phenylmethyl-sulphonylfluo-ride, $CoSO_4$	8.5	50	8.0–9.0	30	[23]
Aspergillus flavus	metalloprotease	23	9.0	elastin, collagen, ovalbumin, albumin	1,10-*o*-phenanthroline, EDTA	7.5	ND	8.0–11.0	<70	[33]
Aspergillus oryzae	chymotrypsin-like	41	ND	hemoglobin	pepstatin, $HgCl_2$	3.0	60	3.0–6.0	<40	[45]
Aspergillus oryzae	keratinase	60	ND	bovine serum albumin, casein, keratin	EDTA, Pb	8.0	50	ND	<70	[18]
Aspergillus ustus (NIOCC #20)	alkaline serine	32	6.6 6.9	azocasein	phenylmethyl-sulphonylfluo-ride, $CuCl_2$	9.0	45	4.5	40	[12]
Aspergillus niger Z1	alkaline	68	ND	azocasein	phenylmethyl-sulphonylfluo-ride	9.0	40	35	<90	[11]

Continued

1	2	3	4	5	6	7	8	9	10	11
Cordyceps militaris	trypsin-type serine protease	23.4	ND	benzyloxycarbonyl; n-benzoyl-phe-val-arg-p-nitroanilide	leupeptin, diisopropylfluo-rophosphate	8.5—12.0	ND	ND	ND	[25]
Chrysosporium keratinophilum	alkaline	69	ND	casein	1,10 *o*-phenanthroline	9.0	90	7.0—10.0	ND	[15]
Penicillium sp. LPB-5	serine	ND	ND	azocasein	phenylmethyl-sulphonyl fluo-ride	6.5	45	6.0—9.0	35—45	[19]
Fusarium culmorum	alkaline serine	28.7	ND	n-succinyl-ala-ala-pro-phep-*p*-nitroanilide, n-succinyl-ala-ala-pro-leup- *p*-nitroanilide, azogelatin	phenylmethane-sulfonylfluoride; chymostatin,	8.3—9.6	40	4.1—7.7	ND	[43]
Sporotrichum pulverulentum	acidic endoprotease 1 acidic protease 2 chymotrypsin-type	28 26	4.7 4.2	azocoll	$AgNO_3$,$HgCl_2$, *p*-chloromercu-ribenzoat	5.0 5.2	ND	ND	ND	[17]
Thermomyces auranticus	protease	ND	ND	casein	ND	5.5	60	3.0-9.5	<50	[34]
Trichoderma reeseisQM9414	trypsine like serine	25	7.3	azocasein, benzoyl-arginyl-p-nitroanilide	ND	8.0	50	ND	ND	[14]

* ND — no date.

protease of isolate (NIOCC # 20) isolated from the sea water (MW 32 kDa, pI 6.6–6.9) was active in broad pH spectrum 6.0–10.0, with optimas at pH 9.0 and temperature 45 °C [12].

Thermostable alkaline protease of *Chrysosporium keratinophilum* was partially purified by acetone precipitation followed by gel filtration on Sephadex G-75 [15]. The optimal conditions for enzyme activity were set at pH 9.0 and temperature 90 °C and protease did not lose activity at pH values from 7.0 to 10.0. The enzyme distinguished by MW 69 kDa was activated by Fe^{2+} and inhibited by 1,10-*o*-phenanthroline.

Myrothecium verrucaria produces protease with atypical for phytopathogenic fungi keratinolytic activity [39]. Crude protease hydrolyzes keratin substrates at pH 9.0 and temperature 40 °C in the following sequence: keratin of fowl feather > keratin of ovine wool > keratin of human hair. Protease activity is sensitive to phenylmethylsulfonylfluoride, allowing to refer it to serine proteases.

Purified keratinase of *Aspergillus oryzae* is a monomeric enzyme having MW 60 kDa [18]. The enzyme hydrolyzed various substrates showing affinity to bovine albumin, casein, keratin, poultry feather keratin, collagen, duck feather, ovine wool. The distinctions in action of immobilized and free enzyme were revealed under the optimal conditions: for immobilized enzyme pH the optimum was lying in the range 7.0–7.4, temperature 60 °C, for free enzyme the optimas were 8.0 and 50 °C, respectively. Keratinase was activated by Ca^{2+} and Ba^{2+} ions and inhibited by EDTA.

Extracellular keratinase-protease of *Scopulariopsis brevicaulis* was purified by sedimentation with ammonium sulfate, chromatography on DEAE-cellulose and Sephadex G-100 [2]. Purified enzyme is a monomeric protein with molecular weight 39 and 36 kDa according to SDS-PAGE and gel-filtration data, respectively. The best conditions for catalysis were established at pH 8.0 and 40 °C.

The analysis of literature reports indicates that fungal proteases differ in substrate specificity, optimal conditions for substrate hydrolysis, pH and thermal stability. Molecular weight of fungal proteases ranges from 15 to 30 kDa, but more heavy-weight enzymes were described: 33 kDa (*Scedosporium apiospermum* protease [30], 41 kDa (*Aspergillus oryzae* protease [45], 62 kDa (*Aspergillus oryzae* protease [18], 68 kDa (*Aspergillus niger* Z1 protease [11], 69 kDa (*Chrysosporium keratinophilum* protease [15]. The variations in optimal conditions for catalysis of degradation of proteinaceous substrates determine broad application scope of fungal proteases.

Applications of fungal proteases

Considering applications of proteolytic enzymes, it should be noted that they have been introduced in commercial flow sheets for manufacturing detergents and cleansing agents, they are widely used in food processing (cheese making, bread baking, beer clarifying, meat tenderizing, producing

protein hydrolyzates), textile and leather industries, medicine, veterinary practice, fodder provision.

Nowadays proteases are the indispensable and traditional components of laundering and cleaning formulas — from domestic washing solutions to the reagents for polishing lenses and dental prostheses [47]. 25% of global enzyme output is channelled to fabrication of detergents. The first enzyme-based product of this type ''Burnus'', containing crude pancreatic extract was launched in 1913, while the first detergent BIO-40 with microbial (bacterial) protease was produced on a large-scale in 1956. In 1960 Novo Industry A/S company started its alkalase project (trade name BIOTEX) and now the market is flooded with various proteolytic preparations. Ideally detergent protease should be distinguished by a broad substrate specificity, activity at elevated pH and temperature values, pH and thermal stability, compatibility with other constituents of cleaning and washing aids. Stringent reality of world economic crisis urges to refocus the emphasis on proteases active at low temperatures. The investigations are currently under way on fungal alkaline proteases for detergents — originating from *Spilosoma obliqua* [4], *Botrytis cinerea* [1], while *Conidiobolus coronatus* serves as a source of detergent protease in India [44].

In leather industry fungal proteases are engaged in 2 processes: hair depilation and skin softening [3, 47].

As to food processing, microbial proteases are the indispensable agents in cheese making, bread baking, brewing, producing hydrolyzates from vegetable and animal materials, meat tenderizing. Proteases of *Mucor michei* and *Endothia parasitica* are manufactured on industrial scale for cheese fermentation [40, 47]. Endo- and exoproteinases of *Aspergillus oryzae* carry out limited proteolytic modification of wheat gluten defining properties and taste of bread [40, 47]. It ensures standardization of baking process and reduces duration of technological cycle. Proteases are widely used to derive diverse products from soya which proved to be an excellent nutrient owing to high protein content. Alkaline and neutral proteases of fungi *Aspergillus oryzae* and *Aspergillus sojae* play a key role in production of soya sauce [46, 47]. Efficiency of protease application was demonstrated for fish sauce cooking technology. The procedure of preparing this flavour, extremely popular in South-Eastern Asia, usually takes from 6 to 12 months. A vital characteristic of proteolytic enzyme intended to accelerate and upgrade the process is its resistance to increased salt concentrations (10—20%) required in soya sauce recipe. The vietnamese researchers have shown that application of *Aspergillus oryzae* protease guarantees yield of soya sauce in just 2—6 days depending on biopreparation level [31]. The increased ratio of free amino acids was recorded in the end product. The attractive prospects were outlined for enzyme preparation of *P. lilacinus* F-2 applied to obtain low-molecular-weight protein hydrolyzates of milk whey as ingredients of enteral and parenteral nutrition products and complex food additives [54].

Correlation of proteolysis degree of major serum proteins: β-lactoglobulin, α-lactalbumin, bovine serum albumin — with concentration of biopreparation was established. At 0.2% enzyme concentration about 72% of β-lactoglobulin and 86% of α-lactalbumin were subjected to proteolysis and bovine serum albumin was efficiently hydrolyzed. Rise in enzyme concentration to 1% resulted in complete splitting of α-lactalbumin to peptides with MW less than 14.2 kDa and only 5% of uncleaved β-lactoglobulin remained in hydrolyzate.

Lately special interest has been focused on the synthesis of proteolytic enzyme preparations due to the problem of utilization of secondary protein resources. Manufacturing of combined and artificial products based on food protein is essential to meet the challenge. Protein hydrolyzates find use as the ingredients of dietetic or therapeutic diets and baby mixes. Hydrolyzate composition largely depends on feedstock preconditioning and type of enzyme preparation. Combination of protein supplements of animal origin with collagen enzymatic hydrolyzate allows to upgrade end products with peptides, amino acids and reduce expense of animal proteins in recipes of preserves and sausages. The balanced protein additives and the products derived from collagen hydrolyzate and vegetable materials have been developed and marketed [6]. The studies were completed on production of hydrolyzates from heads and feet of overland birds [3]. The enzyme preparations from *Penicillium wortmannii* BKM-2091 and *Streptomyces chromogenes s. graecus* 0832 posessing collagenase and keratinolytic activities were recommended for enzymatic treatment yielding protein- lipid emulsion.

Medicine is a vital application sphere for proteolytic enzymes. Neutral protease is essential for therapy of gastrointestinal and cardiovascular diseases, in surgery for treatment of purulent wounds, burns and congealed tissues, in antitumor courses. Proteases of *Aspergillus oryzae* (Luizym and Nortase commercial preparations) are applied in substitution therapy as digestive enzymes [47], while proteases from *Aspergillus oryzae* and *Aspergilus flavus* are the antithrombic agents [40].

Proteolytic enzymes are in great demand by the researchers in the area of physiology, phytopathology and enzymology.

The analysis of presented data evidences prove high theoretical and practical significance of fungal proteolytic enzymes. The wide variety of fungal proteases, their broad substrate specificity, resistance to extreme environmental conditions and multifunctionality point out these enzymes as important objects for further investigations and stress the priority status of mycelial fungi for large-scale production of proteolytic enzymes.

REFERENCES

1. *Abidi F., Limam F., Nejib M.M.* Production of alkaline proteases by *Botrytis cinerea* using economic raw materials: Assay as biodetergent // Process Biochem. — 2008. — Vol. 43. — N. 11. — P. 1202–1208.

2. *Anbu P., Gopinath S.C.B., Hilda A., Lakshmipriya T., Annadurai G.* Purification of keratinase from poultry farm isolate *Scopulariopsis brevicaulis* and statistical optimization of enzyme activity // Enzyme Microb. Technol. – 2005. – Vol. 36. – N 5-6. – P.639–647.

3. *Antipova L.V., Glotova I.A., Rogov I.A.* Methods of analyzing meat and meat products / Moscow, Kolos Press, 2001. – 376 pp.

4. *Anwar A., Saleemuddin* M. Alkaline pH acting digestive enzymes of the polyphagous insect pest *Spilosoma obliqua*: stability and potential as detergent additives // Biotechnol. Appl. Biochem. – 1997. – Vol. 25. – N 1. – P. 43–46.

5. *Bazarzhapov B.B., Lavrent'eva E.V., Dunaevskii Ya.E., Bilanenko E.N., Namsaraev B.B.* Extracellular proteolytic enzymes of microscopic fungi from thermal springs of Barguzin valley (Northern Baikal region) // Appl. Biochem. Microbiol. – 2006. – Vol. 42. – N 2. – P.186–190.

6. *Belousova S.V., Zaporozhsky A.A.* Processing of low-value fish by enzymatic methods // Izvestia vuzov. Pishchevaya technologia. – 2007. – N 3. – P. 48–49.

7. *Böckle B., Galunsky D., Müller R.* Characterization of keratinolytic serine proteinase from *Streptomyces pactum* DSM 40530// Appl. Env. Microbiol. – 1995. – Vol. 61. – N 10. – P. 3705–3710.

8. *Boer C.G., Peralta R.M.* Production of extracellular proteases by *Aspergillus tamarii* // J. Basic Microbiol. – 2000. – Vol. 40. – N 2. – P. 75–81.

9. *Brouta F., Descamps F., Fett T., Losson B., Gerday C., Mignon B.* Purification and characterization of a 43.5 kDa keratinolytic metalloprotease from *Microsporum canis* // Med. Mycol. – 2001. – Vol. 39. – N 3. – P. 269–275.

10. *Chou H., Lai H.Y., Tam M.F., Chou M.Y., Wang S.R., Han S.H., Shen H.D.* cDNA cloning, biological and immunological characterization of the alkaline serine protease major allergen from *Penicillium chrysogenum* // Int. Arch. Allergy Immunol. – 2002. – Vol. 127. – N 1. – P. 15–26.

11. *Coral G., Arican B., Unaldi M.N., Guvenmez H.* Thermostable alkaline protease produced by an *Aspergillus niger* strain // Ann. Microbiol. – 2003. – Vol. 53. – N 4. – P. 491–498.

12. *Damare S., Raghukumar Ch., Muraleedharan D.U., Raghukumar S.* Deep-sea fungi as a source of alkaline and cold-tolerant proteases // Enzym. Microb. Technol. – 2006. – Vol. 39. – N 2. – P. 172–181.

13. *Delgado-Jarana J., Pintor-Toro J.A., Benitez T.* Overproduction of β-1,6-glucanase in *Trichoderma harzianum* is controlled by extracellular acidic proteases and pH // Biochim. Biophys. Acta. – 2000. – Vol. 1481. – N 2. –P. 289–296.

14. *Dienes D., Börjesson J., Hägglund P., Tjerneld F., Lidén G., Réeczey K., Stelbrand H.* Identifcation of a trypsin-like serine protease

from *Trichoderma reesei* QM9414 // Enzyme Microb. Technol. –2007. –Vol. 40. – N 5. – P. 1087–1094.

15. *Dozie I.N.S., Okeke C.N., Unaeze N.C.* A thermostable, alkaline-active, keratinolytic proteinase from *Chrysosporium keratinophilum* // World J. Microbiol. Biotechnol. – 1994. – Vol. 10. – N 5. – P. 563–567.

16. *Dunaevsky Y.E., Gruban T.N., Beliakova G.A., Belozersky M.A.* Enzymes secreted by filamentous fungi: regulation of secretion and purification of an extracellular protease of *Trichoderma harzianum* // Biochemistry (Mosc). – 2000. – Vol. 65. – N 6. – P. 723–727.

17. *Eriksson K.E., Pettersson B.* Acid proteases from *Sporotrichum pulverulentum* // Methods Enzymol. – 1988. – Vol. 160. – P. 500–508.

18. *Farag A.M., Hassan M.A.* Purification, characterization and immobilization of a keratinase from *Aspergillus oryzae* // Enzyme Microb. Technol. – 2004. – Vol. 34. – N 2. – P. 85–93.

19. *Germano S., Pandey A., Osaku C.A., Rocha S.N., Soccol C.R.* Characterization and stability of proteases from *Penicillium sp.* produced by solid-state fermentation // Enzyme Microb. Technol. – 2003. – Vol. 32. – N 2. – P. 246–251.

20. *Gupta R., Beg Q.K., Lorenz P.* Bacterial alkaline proteases: molecular approaches and industrial applications // Appl. Microbiol. Biotechnol. – 2002. – Vol. 59. – N 1. – P. 15–32.

21. *Gzogyan L.A., Proskuryakov M.T., Ievleva E.V., Valueva T.A.* Tripsin-like proteinases and tripsin inhibitors in fruiting bodies of higher fungi // Appl. Biochem. Microbiol. – 2005. – V. 41. – N 6. – P. 538–542.

22. *Haab D., Hagspiel K., Szakmary K., Kubicek C.P.* Formation of the extracellular proteases from *Trichoderma reesei* QM9414 involved in cellulose degradation // J. Biotechnol. – 1990. – Vol. 16. – N 3-4. – P. 187–188.

23. *Hajji M., Rebai A., Gharsallah N., Nasri M.* Optimization of alkaline protease production by *Aspergillus clavatus* ES1 in *Mirabilis jalapa* tuber powder using statistical experimental design // Appl. Microbiol. Biotechnol. – 2008. – Vol. 79. – N 6. – P. 915–923.

24. *Haq I., Mukhtar H., Ali Z., Riaz N.* Protease biosynthesis by mutant strain of *Penicillium griseoroseum* and cheese formation // Pak. J. Biol. Sci. – 2004. – Vol. 7. – N 9. – P. 1473–1476.

25. *Hattori M., Isomura S., Yokoyama E., Ujita M., Hara A.* Extracellular trypsin-like proteases produced by *Cordyceps militaris* // J. Biosci. Bioeng. – 2005. – Vol. 100. – N 6. – P. 631–636.

26. *Ífrij İ.H., Ögel Z.B.* Production of neutral and alkaline extracellular proteases by the thermophilic fungus, *Scytalidium thermophilum*, grown on microcrystalline cellulose // Biotechnol. Lett. – 2002. – Vol. 24. – N 13. –P. 1107–1110.

27. *Jarai G., Buxton F.* Nitrogen, carbon, and pH regulation of extracellular acidic proteases of *Aspergillus niger* // Curr. Genet. – 1994. – Vol. 26. – N 3. – P. 238–244.

28. *Kolattukudy P.E., Lee J.D., Rogers L.M., Zimmerman P., Ceselski S., Fox B., Stein B., Copelan E.A.* Evidence for possible involvement of an elastolytic serine protease in aspergillosis // Infect. Immun. – 1993. – Vol. 61. – N 6. – P.2357-2368.

29. *Lamaison J.L., Pourrat H., Pourratt A.* Purification et propriétés d'une protéase neutre de *Tricholoma columbetta* // Phytochemistry. – 1980. – Vol. 19, N 6. – P. 1021–1023.

30. *Larcher G., Bouchara J.P., Annaix V., Symoens F., Chabasse F., Tronchin G.* Purification and characterization of a fibrinogenolytic serine proteinase from *Aspergillus fumigatus* culture filtrate // FEBS Lett. – 1992. – Vol. 308. – N 1. P. 65–69.

31. *Le Van Viet Man, Tran Thi Anh Tuyet.* Characterization of protease from *Aspergillus oryzae* surface culture and application in fish sauce processing // Sci. Technol. Devel. – 2006. – Vol. 9. – N 5. – P. 53–58.

32. *Li D.-Ch., Yang Y.-J., Shen Ch.-Y.* Protease production by the thermophilic fungus *Thermomyces lanuginosus* // Mycol. Res. – 1997. – Vol. 101. – N 1. – P. 18–22.

33. *Mellon J.E., Cotty P.J.* Purification and partial characterization of an elastinolytic protease from *Aspergilus flavus* culture filtrates // Appl. Microbiol. Biotechnol. – 1996. – Vol. 46. – N 2. – P. 138–142.

34. *Merheb C.W., Cabral H.; Gomes E., Da-SilvaR.* Partial characterization of protease from a thermophilic fungus, *Thermoascus aurantiacus*, and its hydrolytic activity on bovine casein // Food Chem. – 2007. – Vol. 104. – N 1. – P. 127–131.

35. *Mignon B.R., Nikkels A.F., Pierard G.E., Losson B.J.* The in vitro and in vivo production of a 31.5 kD keratinolytic subtilase from *Microsporum canis* and the clinical status in naturally infected cats // Dermatology. – 1998. –Vol. 196. – N 4. – P. 438–441.

36. *Mikhailova I.M., Tazetdinova D.I., Shishkin A.V., Alimova F.K., Skvortsov E.V.* Investigation of protease activity in micromycetes of genus *Trichoderma* // Postgenome era in biology and problems of biotechnology: Proc. II Int. Conf. Kazan, September 15-16, 2008. Kazan, 2008. – P. 86–87.

37. *Monod M., Capoccia S., Léchenne B., Zaugg C., Holdom M., Jousson O.* Secreted proteases from pathogenic fungi // Int. J. Med. Microbiol. – 2002. – Vol. 292. – N 5-6. – P. 405–419.

38. *Monod M., Paris S., Sanglard D., Jaton-Ogay K., Bille J., Latgé J.P.* Isolation and characterization of a secreted metalloprotease of *Aspergillus fumigatus* // Infect. Immun. – 1993. –Vol. 61. – N 10. – P. 4099–4104.

39. *Moreira F.G., de Souza C.G.M., Costa M.A.F., Reis S., Peralta R.M.* Degradation of keratinous materials by the plant pathogenic fungus

Myrothecium verrucaria // Mycopatologia. – 2007. – Vol. 163. – N 3. – P. 153–160.

40. *North M.J.* Comparative biochemistry of the proteinases of eucariotic microorganisms // Microbiol. Rev. – 1982. – Vol. 46. – N 3. – P. 308–340.

41. *Ogundero V.W., Osunlaja S.O.* The purification and activities of an alkaline protease of *Aspergilus clavatus* from Nigerian poultry feeds // J. Basic Microbiol. – 1986. – Vol.26. – N 4. – P. 241–248.

42. *Omkolthoum A.G.A.* Formation of proteases by *Aspergillus fumigatus* and *Penicillium sp.* // J. King Saud University. Science. – 1992. – Vol. 4. – N 2. – P. 127–136.

43. *Pekkarinen A.I., Jones B.L., Niku-Paavola M.L.* Purification and properties of an alkaline protease of *Fusarium culmorum* // Eur. J. Biochem. – 2002. – Vol. 269. – N 3. – P. 798–807.

44. *Phadatare S.U., Deshpande V.V., Srinivasan M.C.* High activity alkaline protease from *Conidiobolus coronatus* (NCL 86.8.20): enzyme production and compatibility with commercial detergents // Enzyme Microb. Biotechnol. – 1993. – Vol. 15. – N 1. – P. 72–76.

45. *Pong C.-Y., Chou Y.-H., Nieh F.-P., Jiang S.-T.* Purification and characterization of acidic protease from *Aspergillus oryzae* // J. Fish. Soc. Taiwan. – 2005. – Vol. 32. – N 3. – P. 253–264.

46. *Priest F.* Extracellular enzymes / F. Priest / Moscow, Mir Publ., 1987. 117pp.

47. *Rao M.B., Tanksale A.M., Ghatge M.S., Deshpande V.V.* Molecular and biotechnological aspects of microbial proteases // Microbiol. Mol. Biol. Rev. – 1998. – Vol. 62. – N 3. – P. 597–635.

48. *Reichard U., Büttner S., Eiffert H., Staib F., Rüchel R.* Purification and characterization of an extracellular serine proteinase from *Aspergillus fumigatus* and its detection in tissue // J. Med. Microbiol. – 1990. – Vol. 33. – N 4. – P.243–251.

49. *Santos R.M.D.B., Firmino A.A.P., de Sá C.M., Felix C.R.* Keratinolytic activity of *Aspergillus fumigatus* Fresenius // Curr. Microbiol. – 1996. – Vol. 33. – N 6. – P. 364–370.

50. *Sekine H.* Neutral proteinases I and II of *Aspergillus sojae*: some enzymatic properties // Agric. Biol. Chem. – 1972. – Vol. 36. – N 2. – P. 207–216.

51. *Shrinivasan M.C., Vartak H.G., Powar V.K., Sutar I.I.* High activity alkaline protease production by a *Conidiobolus* SP // Biotechnol. Lett. – 1983. – Vol. 5. – N 5. – P. 285–288.

52. *Tremacoldi C.R., Carmona E.C.* Production of extracellular alkaline proteases by *Aspergillus clavatus* // World J. Microbiol. Biotechnol. – 2005. – Vol. 21. – N 2. – P. 169–172.

53. *Tsirkunova Zh.F., Chikhaeva O.V., Mikhailova R.V., Lobanok A.G.* Screening of mycelial fungi producing proteolytic enzymes // Proc. VI Int Conf. «Contemporary state and development prospects of microbiology and

biotechnology», Minsk, June 2-6, 2008. – Minsk, Loginov Press, 2008. – V. 1. – P. 276–278.

54. *Tsirkunova Zh.F., Mikhailova R.V., Lobanok A.G.* Properties of extracellular proteases from *Penicillium lilacinum* F-2 // XII Congress microbiol. Ukraine: Abstr. reports / Uzhgorod, Patent Publ. 2009. – P. 412.

55. *Tsuchiya K., Arai T., Seki K., Kimura T.* Purification and some properties of alkaline proteinase from Cephalosporium sp. KM388 // Agric. Biol. Chem. – 1987. – Vol. 51. – N 11. – P. 2959–2965.

56. *Tunga R., Banerjee R., Bhattacharyya B.* Optimizing some factors affecting protease production under solid state fermentation // Bioprocess Eng. – 1998. – Vol. 19. – N 3. – P. 187–190.

57. *Tunlid A., Rosén S., Ek B., Rask L.* Purification and characterization of an extracellular serine protease from the nematode-trapping fungus *Arthrobotrys oligospora* // Microbiol. – 1994. – Vol. 140. – No.pt 7. – P. 1687–1695.

58. *Wang L., Ridgway D., Gu T., Moo-Young M.* Bioprocessing strategies to improve heterologous protein production in filamentous fungal fermentations // Biotechnol. Advances. – 2005. – Vol. 23. – N 2. – P. 115–129.

59. *Wang S-L., Chen Y-H., Wang C-L., Yen Y-H., Chern M-K.* Purification and characterization of serine protease extracellularly produced by *Aspergillus fumigatus* in a shrimp and crab shell powder medium // Enzyme Microb. Technol. – 2005. — Vol. 36. — N 5-6. — P. 660–665.

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ПРОТЕОЛІТИЧНІ ФЕРМЕНТИ МІЦЕЛІАЛЬНИХ ГРИБІВ

Реферат

Наведені літературні і власні експериментальні дані з утворення протеолітичних ферментів міцеліальними грибами, властивостей протеаз і їх застосування. Показана широка розповсюдженність здатності синтезувати протеази серед грибів різних родів. Грибні протеази відрізняються субстратною специфічністю, мають широкий діапазон значень оптимальних умов гідролізу субстрату, pH- і термостабільності. Множинні аспекти застосування грибних протеаз та їх каталітичні властивості обумовлюють перспективність грибів як промислових продуцентів зазначених ферментів.

Ключові слова: міцеліальні гриби-продуценти, протеолітичні ферменти, властивості, гідроліз, застосування.

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ПРОТЕОЛИТИЧЕСКИЕ ФЕРМЕНТЫ МИЦЕЛИАЛЬНЫХ ГРИБОВ

Реферат

Приведены литературные и собственные экспериментальные данные по образованию протеолитических ферментов мицелиальными грибами, свойствам протеаз и их применению. Показана широкая распространенность способности синтезировать протеазы среди грибов различных родов. Грибные протеазы отличаются субстратной специфичностью, имеют широкий диапазон значений оптимальных условий гидролиза субстрата, pH- и термостабильности. Множественные аспекты применения грибных протеаз и их каталитические свойства обуславливают перспективность грибов как промышленных продуцентов указанных ферментов.

Ключевые слова: мицелиальные грибы-продуценты, протеолитические ферменты, свойства, гидролиз, применение.