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EXPERIMENTAL ARTICLES

Extracellular Hydrolases of Strain *Bacillus* sp. 739 and Their Involvement in the Lysis of Micromycete Cell Walls

G. E. Aktuganov[1], N. F. Galimzyanova, A. I. Melent'ev, and L. Yu. Kuz'mina

Institute of Biology, Ufa Research Center, Russian Academy of Sciences

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Abstract—The mycolytic bacterial strain *Bacillus* sp. 739 produces extracellular enzymes which degrade in vitro the cell walls of a number of phytopathogenic and saprophytic fungi. When *Bacillus* sp. 739 was cultivated with *Bipolaris sorokiniana*, a cereal root-rot pathogen, the fungus degradation process correlated with the levels of the β-1,3-glucanase and protease activity. The comparative characteristic of *Bacillus* sp. 739 enzymatic preparations showed that efficient hydrolysis of the fungus cell walls was the result of the action of the complex of enzymes produced by the strain when grown on chitin-containing media. Among the enzymes of this complex, chitinases and β-1,3-glucanases hydrolyzed most actively the disintegrated cell walls of *B. sorokiniana.*. However, only β-1,3-glucanases were able to degrade the cell walls of native fungal mycelium in the absence of other hydrolases, which is indicative of their key role in the mycolytic activity of *Bacillus* sp. 739.

Key words: mycolytic enzymes, *Bacillus* spp., chitinase, β-1,3-glucanase, antagonism, phytopathogenic fungi.

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Aerobic spore-forming bacteria are among the best-studied and promising biological subjects for the development of the modern means to protect the cultivated plants against diseases caused by phytopathogenic fungi [1–5]. The main features of bacilli that make them attractive agents of biological control of phytopathogens are their wide prevalence, the capacity for rapid growth and endospore formation, relative safety for humans and animals, as well as the production of a broad spectrum of biologically active compounds [6, 7]. The ability of many strains of *Bacillus* to colonize the rhizosphere of cultivated plants and stimulate their growth is of great importance in this respect [8]. *Bacillus* sp. 739, which is used as a biological basis for the experimental preparation Batsispetsin BM, effective against fungal pathogenic agents of cereal diseases, is among such strains [9]. The efficacy of this strain as a biocontrol agent is determined not only by its antagonistic activity but, probably also by the production of substances of a phytohormonal nature [10]. Earlier, the ability of *Bacillus* sp. 739 to colonize wheat rhizosphere (*Triticum aestivum* L.) was demonstrated by electron microscopy [11]. These results, together with other data, allow us to regard this strain as a representative of plant-growth–promoting rhizobacteria (PGPR).

Understanding of the functioning of various mechanisms of antagonistic action and the conditions of their realization remains a topical problem in present-day studies determining the positive effect of preparations based on bacterial antagonists. The synthesis of extracellular hydrolases capable of destroying fungal cell-wall structural polymers is considered to be one of the possible mechanisms. Among the mycolytic enzymes, chitinases (EC 3.2.1.14) and β-1,3-glucanases (EC 3.2.1.6; 3.2.1.39), which are quite widespread among the representatives of saprophytic soil microflora, attract the most attention. However, the active involvement of enzymes in inhibiting the growth of phytopathogenic fungi has been confirmed only for individual well-studied groups of microbial antagonists [12]. The role of extracellular hydrolases of most saprophytic antagonistic bacteria in the biological control of phytopathogens is poorly studied, since their main function is supposed to be the decomposition of organic matter from the dead fungal mycelium for nutrient extraction [13]. The involvement of chitinase in the antagonistic interaction with phytopathogenic fungi has been confirmed for a number of gram-negative bacteria [13]. However, the data on the antifungal function of the *Bacillus* extracellular hydrolases need to be specified. The assessment of the antifungal activity of the purified exochitinase of *Bacillus* sp. strain 739 in one of our previous works yielded a negative result [14]. Nevertheless, in vitro studies of the biological properties of individual enzyme fractions do not always take into account the complex nature of microbial antagonism and the mycolytic process (which imply the interaction between different enzyme groups), or of the enzymes proper and secondary metabolites [12, 13, 15]. Therefore, the conclusions about the role of lytic

[1] Corresponding author; e-mail: gleakt@anrb.ru.

enzymes in antagonism of bacilli cannot be based solely on assessing the activity of chitinases, especially represented by one or several isoforms with similar characteristics. This is also confirmed by the fact that a number of aerobic spore-forming bacteria exhibited correlation between the antagonistic activity against various pathogenic fungi and the synthesis of hydrolytic enzymes such as cellulose, xylanase, mannanase, and protease [6].

Earlier, we discovered that, when cultivated in a liquid medium containing sterile mycelium or isolated fungal cell walls, *Bacillus* sp. strain 739 produces a complex of glycosyl hydrolases and proteases involved in the degradation of this substrate [16]. The aim of the present work was to assess the lytic effect of these enzymes on the disintegrated cell-wall material and the native mycelium of a number of phytopathogenic and saprophytic micromycetes, as well as to assess their involvement in the antagonistic interaction between *Bacillus* sp. 739 and the fungus *Bipolaris sorokiniana*.

MATERIALS AND METHODS

The work used the strain *Bacillus* sp. 739 deposited in the collection of the All-Russian Research Institute of Agricultural Microbiology under the number 132 [9]. The strain was maintained on solid nutrient medium of the following composition (g/l): colloidal chitin, 5.0; peptone, 1.0; yeast extract, 1.0; corn extract, 0.5; $(NH_4)_2HPO_4$, 1.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; $CaCl_2$, 0.3; agar, 16.0 (pH after autoclaving 6.5 ± 0.2).

The cereal root rot pathogen *Bipolaris sorokiniana* (Sacc.) Shoemaker (=*Helminthosporium sativum* Pam., King et Bakke or *Drechlera sorokiniana* (Sacc.) Subram. et gain.), isolated and identified at the Institute of Biology, Ufa Research Center, Russian Academy of Sciences, was the main test object in the work [17]. Several species of phytopathogenic and saprophytic fungi from the All-Russian Collection of Microorganisms and the collection of the Institute of Biology, Ufa Research Center, Russian Academy of Sciences, were used, namely, *Fusarium culmorum* (W. G. Smith) Sacc. VKM F-844, *F. graminaerum* Schwabe VKM F-1668, *Rhizoctonia solani* Kuhn VKM F-935, *Alternaria alternata* (Fr.) Keissl. IB-1, *Aspergillus niger* van Tieghem IB-1, *Paecilomyces variotii* Bainier IB-1, and *Trichoderma* sp. Pers IB-2. In order to obtain intact mycelium, the fungi were grown under static conditions in the potato–glucose broth at 28°C for 10–14 days. After cultivation, the mycelium was washed twice with 0.9% NaCl to remove the culture medium residues and stored in this solution at 5–10°C. The washed and dried mycelium was further used for obtaining disintegrated cell-wall material [16].

The *B. sorokiniana* culture, along with other micromycetes, was maintained on Czapek agar and on potato–glucose agar (PGA). Combined cultivation of the antagonistic strain with *B. sorokiniana* was carried out in two-fold diluted standard potato medium without glucose.

To assess the inhibition of *B. sorokiniana* growth in a mixed culture, the nutrient medium was inoculated with fungal spore and hypha suspension and the 18–20-h *Bacillus* sp. 739 culture; the latter was introduced into the medium simultaneously with the fungus (0-h culture) or 24 h after inoculation (24-h culture). In the latter variant, preliminary cultivation of the fungus was carried out under static conditions at 28°C. The bacterial strain was preliminarily grown at 37 ± 0.5°C on a shaker at 170 rpm in the medium of the following composition (g/l): potato starch, 5.0; peptone, 2.0; yeast extract, 1.0; corn extract, 0.5; $(NH_4)_2HPO_4$, 1.0, KH_2PO_4, 1.0, $MgSO_4 \cdot 7H_2O$, 0.5. The bacterial cells were then washed off the culture medium residues by centrifugation in a sterile NaCl solution (0.9%). The washed biomass was resuspended in the same solution; 1 ml of the suspension was added per 50 ml of potato medium (the titer $\geq 10^6$ CFU). The *B. sorokiniana* inoculum was prepared by suspending the sporulating fungal culture in sterile 0.9% NaCl. The titer of the *B. sorokiniana* conidia introduced into the medium was $\leq 10^4$.

The mixed culture was incubated under static conditions in 250-ml flasks at 28 ± 0.5°C for eight days. The growth of *B. sorokiniana* was assessed after 1, 2, 4, 6, and 8 days of incubation. The mycelium was washed with distilled water on a Buchner funnel using preliminarily weighed Whatman 1 filters through which bacterial cells were able to pass. The washed mycelium was dried at 105°C to attain a constant dry-mass value. The mycelium yield in the control variant was taken as 100%. The degree of fungal growth inhibition was expressed as a percentage difference between the control and experimental variants. All the experiments were made in triplicate.

The activity of the chitinolytic enzymes was assessed by the rate of formation of reducing sugars (products of hydrolysis of colloidal chitin and chitosan) [16]. The activity of N-acetyl-β-glucosaminidase was measured by the method described in [16]. The β-1,3-glucanase activity was assessed using a 0.2% (wt/vol) laminarin solution as substrate (Sigma, United States) [16]. The cellulase (endo-1,4-glucanase) activity was assessed by changes in the viscosity of 0.5% Na-carboxymethyl cellulose (CMC) solution (medium viscosity, lot C4888, Sigma, United States) in the reaction mixture [18]. The endo-1,4-β-xylanase, pullulanase, and dextranase activities were determined by the formation of the reducing reaction products; the enzyme complex was incubated with 1.0% solutions of the respective substrates at pH 6.0 and 50°C for 10–60 min. Soluble xylan from birch wood pulp (Sigma, United States), pullulan from *Aureobasidium pullulans* (Sigma, United States), and dextran T500 (Pharmacia, Sweden) were used as substrates. The protease and lipase activity of *Bacillus* sp. 739 was measured using

Table 1. Activity of the extracellular hydrolases of *Bacillus* sp. strain 739 grown on media with different substrates

Enzymatic activity, U ml^{-1} CL	Substrate, 0.5% (wt/vol)		
	starch	colloidal chitin	*Fusarium culmorum* mycelium
Chitinase	0.02 ± 0.005	0.21 ± 0.03	0.16 ± 0.02
Chitosanase	0.01 ± 0.005	0.11 ± 0.02	0.14 ± 0.02
N-acetyl-β-glucosaminidase	ND	1.50 ± 0.11	1.67 ± 0.12
Endo-β-1,3-glucanase	3.47 ± 0.25	4.70 ± 0.32	5.95 ± 0.35
Endo-β-1,4-glucanase	–	–	–
Endo-β-1,4-xylanase	0	0	0
Protease	0.70 ± 0.10	0.75 ± 0.10	1.6 ± 0.15
Dextranase	0	0	0
Pullulanase	0	0	0
Lipase	1.45 ± 0.15	1.70 ± 0.15	4.55 ± 0.30

Notes: ND stands for "not determined"; a dash in a number of columns indicates that a reliable estimate of the enzymatic activity was impossible using the methods available. The strain was cultivated on a shaker (170 rpm) at 36.5 ± 0.5°C for 72h.

the methods described in [19]. The enzyme amount catalyzing the formation of 1 μM-equiv of the corresponding monomer in 1 min in 1 ml of the reaction mixture under the conditions described was taken to be a unit activity [16]. The protease activity was expressed in units of commercial proteinase K (Sigma, United States) activity calculated from the calibration graph using azocasein as substrate (Sigma, United States) [19]. The enzyme amount catalyzing the release of 1 μM of *p*-nitrophenol when hydrolyzing *p*-nitrophenyl butyrate (Sigma, United States) in 1 min at 37°C was taken to be a lipase activity unit [19]. The fractionation and purification of the main enzymes of the *Bacillus* sp. 739 lytic complex were carried out using the methods of salt precipitation, anion-exchange chromatography on DEAE-Sephadex A-50, affinity adsorption on chitin, and gel chromatography on Biogel P-100 [14, 20].

The micromycete cell wall hydrolysis by the enzyme complex of *Bacillus* sp. 739 culture liquid (CL) and by the individual enzymes was assessed by incubating intact (5% wt/wt) or ground (1% sol/wt) mycelium in the phosphate–citrate buffer (pH 6.0, 50 mM) containing CL or a diluted sample of the enzyme studied. The reaction was carried out at 37°C for 6–8 h. Sampling was carried out every 2 h to determine the concentration of reducing sugars using the ferricyanide method [18]. The activity was expressed in μM-equiv of free N-acetyl-D-glucosamine per 1 ml in 1 min.

For visual assessment of the action of *Bacillus* sp. 739 on *Bipolaris sorokiniana* in a mixed culture, an Amplival 30-G048a microscope (Carl Zeiss Jena, Germany) was used. Differential staining of the fungal cell walls was carried out using the universal method [21].

The experiments were conducted in triplicate; the statistical data processing was performed with Student's *t*-test for the 5% level of significance.

RESULTS AND DISCUSSION

An increased synthesis of the specific hydrolases involved in the degradation of the cell walls of phytopathogenic fungi by strain *Bacillus* sp. 739 was observed in the medium containing colloidal chitin, as well as under the conditions simulating an interaction with the fungus, when the autoclaved mycelium of phytopathogenic fungi and the ground biomass of basidiomycetes were introduced into the medium [16]. The data presented in Table 1 show that, among the hydrolases tested, the activity of chitinolytic and glucanolythic enzymes, as well as that of proteases and lipases, increased to the highest degree. The level of secretion of chitinolytic enzymes in the monoculture of *Bacillus* sp. 739 depended mainly on the content of specific substrates in the nutrient medium. At the same time, in the presence of substrates of different nature, the strain exhibited stable synthesis of β-1,3-glucanase, whose activity varied within rather narrow limits and depended mainly on the bacterial culture growth parameters (Table 2). A significant decrease in the β-1,3-glucanase synthesis was detected only in the presence of glucose; it can probably be explained by catabolic repression (Table 2). This fact shows that the standard conditions for growth of the fungi may not contribute to the elevated production of the main myc-

Table 2. Level of the β-1,3-glucanase activity in *Bacillus* sp. 739 grown with inducers of different nature

Substrate*	β-1,3-Glucanase activity in CL	
	U ml^{-1}	%
Basal carbohydrate-free medium**	2.83 ± 0.19	100
Starch	3.47 ± 0.25	123 ± 8
Pullulan	2.27 ± 0.13	80 ± 4.6
Inulin	4.40 ± 0.32	155 ± 11
Na-CMC	2.40 ± 0.15	85 ± 5
Pectin	0.44 ± 0.05	15 ± 1.8
D-glucose	0.17 ± 0.03	6 ± 1.1
N-acetyl-*D*-glucosamine	3.25 ± 0.22	115 ± 7.8
Lactose	4.94 ± 0.35	175 ± 12.4
Galactose	5.11 ± 0.35	181 ± 12.4
B. sorokiniana mycelium***	1.26 ± 0.15	44 ± 5

Notes: * The substrate concentration in the experiment is 0.5% mass.

** The basal medium composition, including the minerals and the organic nitrogen sources, is cited in the experimental part.

*** In this variant, the *B. sorokiniana* mycelium was the only source of carbon and nitrogen in tap water without mineral additives.

olytic enzymes by *Bacillus* sp. 739 in mixed culture. The preliminary assessment of the influence of the glucose concentration in the medium on the *B. sorokiniana* biomass yield and on β-1,3-glucanase synthesis by *Bacillus* sp. 739, when they were cultivated separately,

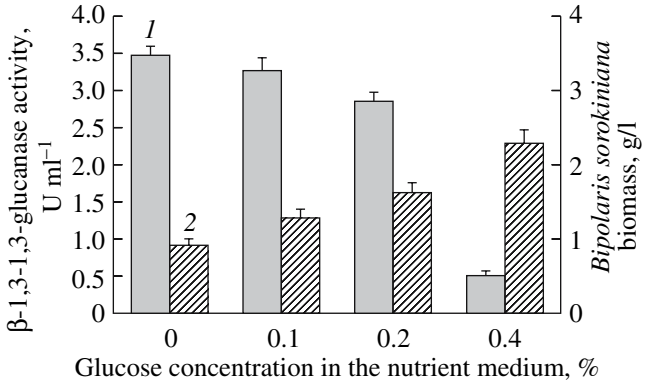

Fig. 1. Influence of the content of D-glucose as an additional carbon source in the basal medium on (*1*) β-1,3-glucanase production by *Bacillus* sp. 739 and (*2*) the growth of *Bipolaris sorokiniana* when they were cultivated separately (the duration of the experiment is four days).

confirmed the presence of an inverse relationship between these parameters (Fig. 1). Combined cultivation was therefore carried out in the twofold diluted standard potato medium without exogenous sugars.

B. sorokiniana revealed the highest sensitivity to the action of the antagonist when they were introduced into the nutrient medium simultaneously (0-h culture, Fig. 2). The lytic effect of the bacterial culture on the fungus increased during incubation (Fig. 3). While after one or two days of incubation this was manifested by the local lysis of the cell walls of the fungal hyphae, further reproduction of bacterial cells resulted in a complete loss of the structural integrity of fungal cell walls, resulting in the total degradation of the mycelium (Fig. 3). A weaker lytic effect was revealed by the antagonist introduced into the 24-h *B. sorokiniana* culture; the degree of inhibition of the fungal growth was 65–75% compared to the control (Fig. 2). The action of the respective bacterial hydrolases seemed to be the most likely mechanism of this; however, no induction of the chitinolytic enzymes of *Bacillus* sp. 739 was revealed in the course of the incubation period. This fact may be due to the specific features of the *B. sorokiniana* cell wall structure, since in many representatives of asco- and basidiomycetes, chitin is known to be present only in the cell wall internal layer, which is inaccessible to chitinases [22]. Among other enzymes in the mixed culture, we revealed only the β-1,3-glucanase and protease activities, which increased in parallel to the dynamics of the fungal mycelium degradation, peaking after six to eight days (Figs. 3, 4). At a similar level of β-1,3-glucanase activity in the medium (attained by the end of the incubation), the 24-h fungal culture underwent only partial lysis, which is likely the result of the presence of more mature hyphae resistant to the action of the lytic factors of the antagonist. The conclusion concerning the bacterial nature of the β-1,3-glucanase activity was based on the fact that it was not revealed in the *B. sorokiniana* monoculture, whereas the activity of the bacterial strain under similar conditions and in the absence of the fungus constituted 20–25% of the level of activity in the experimental variant (Fig. 4). Unlike the β-1,3-glucanase activity, the protease activity in the mixed culture of the antagonist and *B. sorokiniana* (0 h and 24 h) fluctuated within approximately the same level for six days. However, by the eighth day the protease activity in the 24-h culture was twice as high as in the 0 h culture. This phenomenon in no way correlated with the mycelium degradation rate. An increase in the proteolytic activity of the fungus itself due to the mechanisms of resistance to the action of the antagonist and its metabolites is one of its known explanations [23]. However, it does not fully exclude the involvement of the *Bacillus* sp. 739 proteases in the lysis of the *B. sorokiniana* mycelium at the initial stage of their interaction, when the dynamics of changes for both parameters was of a similar character (Figs. 3, 4).

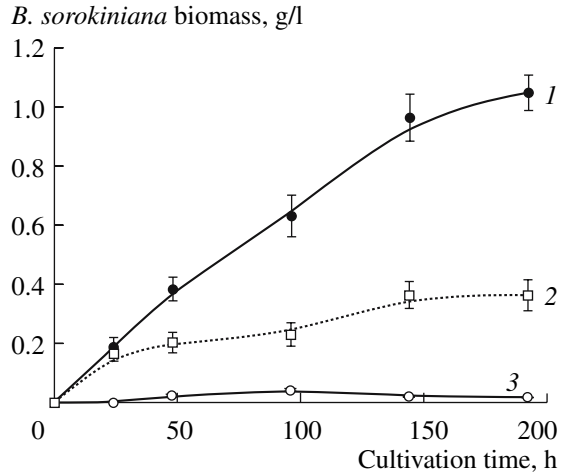

Fig. 2. Dynamics of growth of the *B. sorokiniana* biomass in a mixed culture with *Bacillus* sp. 739, depending on the time the nutrient medium was coinoculated with the antagonist: (*1*) *B. sorokiniana* (control); (*2*) 24 h *B. sorokiniana* + *B subtilis* sp. 739; (*3*) 0 h *B. sorokiniana* + *B subtilis* sp. 739.

The limited synthesis of the mycolytic enzymes by *Bacillus* sp. 739 upon interaction with *B. sorokiniana*, compared to the antagonist monoculture (Table 1, Fig. 4), may be explained by the specific cultivation conditions such as lack of nutrients and the absence of active aeration and of specific substrates. The results obtained show that under the conditions described, the viable mycelium of *B. sorokiniana* is not an effective inducer of *Bacillus* sp. 739 chitinases because the amounts of the fungal biomass introduced do not provide for the synthesis of these enzymes at a measurable level. These conclusions necessitated assessing the real mycolytic activity of the main *Bacillus* sp. 739 enzymes induced in media with colloid chitinal or fungal biomass.

The study of the action of the *Bacillus* sp. 739 hydrolase complex showed its effectiveness in the lysis of the native mycelium of different species of phytopathogenic and saprophytic fungi (Table 3). Of the purified *Bacillus* sp. 739 enzymes tested, β-1,3-glucanases and chitinases most actively degraded the disintegrated cell-wall material of *B. sorokiniana* (Fig. 5). However, when the intact mycelium of different fungi was tested, the hydrolytic activity of β-1,3-glucanases increased approximately 5–10-fold compared to the

Table 3. Rate of hydrolysis of the cell walls of the intact mycelium of various micromycetes by the complex preparation of *Bacillus* sp. 739 lytic enzymes

Test object	GlcNAc release rate, μg-equiv GlcNAc /(ml h)$^{-1}$			
	2 h	4 h	6 h	Average
Alternaria alternata	9.5 ± 1.1	11.3 ± 1.5	15.2 ± 1.7	12.0
Aspergillus niger	4.5 ± 0.5	20.8 ± 1.9	28.3 ± 2.5	17.8
Bipolaris sorokiniana	23.5 ± 2.5	17.0 ± 2.0	31.2 ± 3.5	24.0
Fusarium culmorum	7.5 ± 0.6	6.8 ± 0.5	11.2 ± 1.5	8.5
F. graminearum	4.5 ± 0.5	7.5 ± 0.8	8.8 ± 0.9	6.9
Paecilomyces variotii	22 ± 1.9	25.8 ± 3.0	27.7 ± 3.0	25.2
Rhizoctonia solani	96 ± 8.0	150.8 ± 12.5	161.2 ± 15.0	136.0
Trichoderma sp.	49 ± 5.5	65.3 ± 7.5	64.2 ± 6.5	59.5

Notes: * The concentration of the main lytic enzymes in the reaction mixture: chitinase, 0.15 U ml^{-1}; chitosanase, 0.10 U ml^{-1}; β-1,3-glucanase, 2.0 U ml^{-1}; protease, 0.35 U ml^{-1}.

** The rate of release of reducing sugars was recorded every 2 h of incubation at 37°C and pH 6.0

Table 4. Rate of hydrolysis of the cell walls of the intact mycelium of some phytopathogenic fungi by the main enzymes of the mycolytic complex of *Bacillus* sp. 739 after their purification

Purified enzyme fraction, U ml^{-1}	GlcNAc release rate, μg-equiv (ml h)$^{-1}$		
	Rhizoctonia solani	*Bipolaris sorokiniana*	*Alternaria alternata*
Chitinase, 0.15	5.5 ± 0.6	1.8 ± 0.4	1.3 ± 0.4
β-1,3-glucanase, 0.45	23.2 ± 1.7	13.8 ± 0.8	15.2 ± 0.9
Mixture of both enzymes (1 : 1)	26.4 ± 1.8	18.0 ± 1.2	17.2 ± 0.9

Note: The rate of hydrolysis of the fungal cell walls was calculated as the average rate of formation of mg-equiv of N-acetyl-*D*-glucosamine (GlcNAc) during 8 h of incubation of the reaction mixture at 37°C.

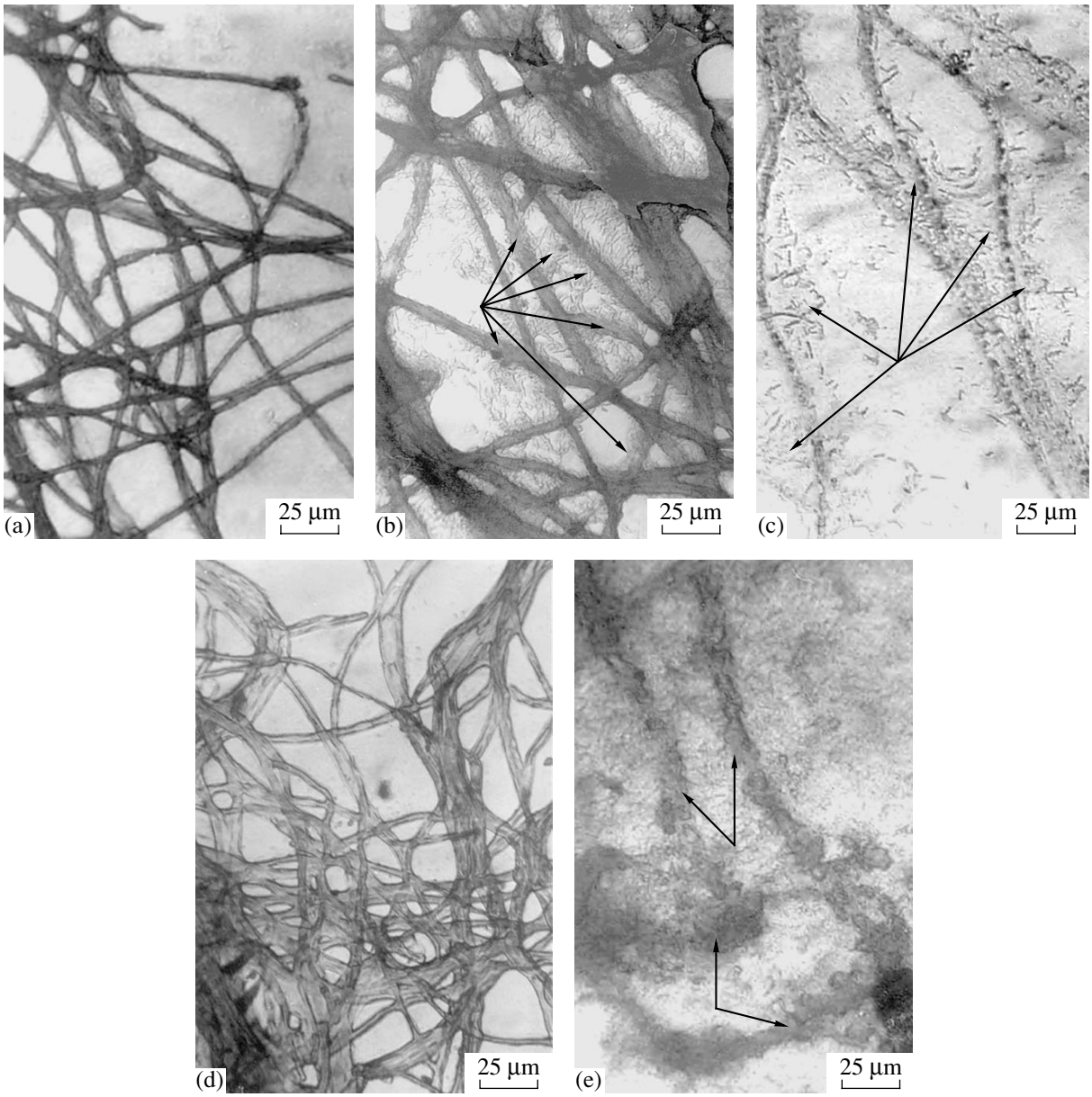

Fig. 3. Degradation of *B. sorokiniana* hyphae by *Bacillus* sp. strain 739 on the simultaneous inoculation of the potato medium with both cultures. The arrows show (b) local degradation of the cell walls (48 h), (c) the sites of mass reproduction of bacterial cells (96 h), and (e) structured hyphal residues after the mycelium mass lysis (144 h). Control: (a) 48 h and (d) 144 h.

activity of chitinases (Table 4). Chitosanase was the least active among the chitinolytic enzymes (Fig. 5). Part of the hydrolytic action of this enzyme on the disintegrated cell walls of *B. sorokiniana* may be explained by its nonspecific chitin-degrading activity, which was experimentally confirmed earlier [20]. Thus, the key role of β-1,3-glucanases in the lysis of the *B. sorokiniana* mycelial cell walls by the *Bacillus* sp. 739 enzyme complex was confirmed in the experiments in vitro. The results obtained indicate a substantial difference in the functional characteristics of the extracellular hydrolases of *Bacillus* sp.739 upon their antagonistic interaction with the fungi.

The capacity of *Bacillus* sp.739 for active growth in media containing chitin or fungal biomass testifies to the high mycolytic potential of this strain, which, in part, determines its antagonistic activity against phytopathogenic fungi [9]. The utilization of the chitin-containing substrate by the strain in question is coupled with its production of the complex of specific hydrolases that includes chitinases, chitosanases, β-1,3-glucanases, and proteases. Among these enzymes, exochitinases and β-1,3-glucanases revealed the highest activity in hydrolyzing the disintegrated cell walls of the phytopathogenic fungus *B. sorokiniana*. However, it seems that the complex of

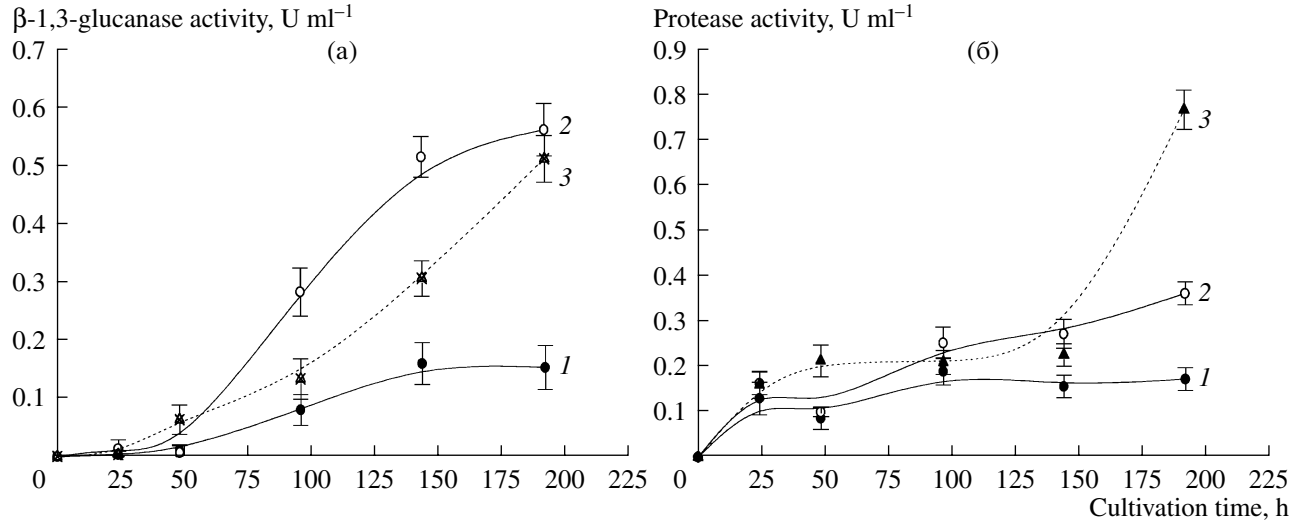

Fig. 4. Changes (a) in the β-1,3-glucanase and (b) protease activity in a mixed culture of *B. sorokiniana* and *Bacillus* sp. 739, depending on the bacterial strains' coinoculation time: (*1*) *Bacillus* sp. 739 (control); (*2*) 0 h *B. sorokiniana* + *Bacillus* sp. 739; (*3*) 24 h *B. sorokiniana* + *Bacillus* sp. 739.

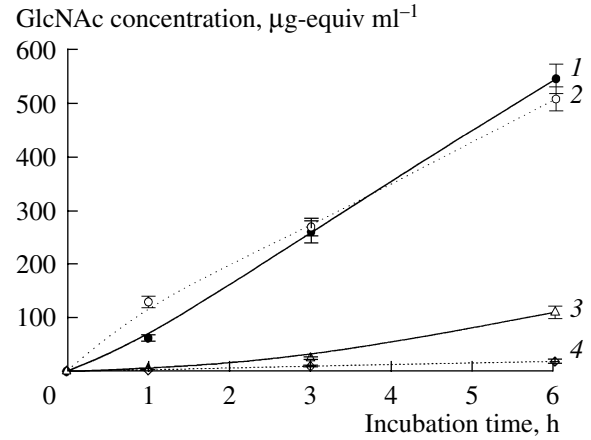

Fig. 5. Dynamics of hydrolysis of the polymers of *B. sorokiniana* disintegrated cell walls (1% sol/wt) by the purified enzymes of the *Bacillus* sp. 739 mycolytic complex. (*1*) chitinase, 0.15 U ml^{-1}; (*2*) β-1,3-glucanase, 0.45 U ml^{-1}; (*3*) chitosanase, 0.12 U ml^{-1}; (*4*) protease, 0.10 U ml^{-1}.

β-1,3-glucanases, which exhibited the highest activity in the lysis of the intact fungal mycelium, should be considered the key component of the *Bacillus* sp.739 lytic system (Table 4). The constitutive synthesis of β 1,3-glucanases at low glucose concentrations along with proteases, implies the presence in *Bacillus* sp. 739 of an inherent lytic factor as one of the mechanisms of antagonistic interaction with micromycetes, which is confirmed by the strain retaining its high mycolytic potential under various conditions of its cultivation.

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