ISSN 0026-2617, Microbiology, 2008, Vol. 77, No. 6, pp. 700–709. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © G.E. Aktuganov, A.I. Melent'ev, N.F. Galimzyanova, A.V. Shirokov, 2008, published in Mikrobiologiya, 2008, Vol. 77, No. 6, pp. 788–797.

EXPERIMENTAL ARTICLES

The Study of Mycolytic Properties of Aerobic Spore-Forming Bacteria Producing Extracellular Chitinases

G. E. Aktuganov[1], A. I. Melent'ev, N. F. Galimzyanova, and A. V. Shirokov

Institute of Biology, Ufa Research Center, Russian Academy of Sciences, Pr. Oktyabrya, 69, Ufa, 450054 Russia

Received October 30, 2007; in final form, April 3, 2008

Abstract—The mycolytic activity of 27 strains of antagonistic bacilli belonging to two taxonomic groups (18 strains of *Bacillus subtilis* and 9 strains of *Paenibacillus ehimensis*) capable of induced synthesis of chitinolytic enzymes was studied. Most of the *B. subtilis* strains neither displayed visible mycolytic effects on the phytopathogenic fungus *Bipolaris sorokiniana* in vitro, nor produced chitinases in the presence of an autoclaved mycelium. On the contrary, *P. ehimensis* strains grown under conditions favorable for induction of chitinases and other hydrolases exhibited a pronounced lytic effect on *B. sorokiniana* and actively grew by utilizing mycelium as the sole source of carbon and nitrogen. Comparison of the mycolytic activities of extracellular hydrolases in the studied strains demonstrated low correlation between chitinase production and the ability of the strains to degrade the cell walls of *B. sorokiniana.* Characterization of enzyme profiles in the studied strains revealed that β-1,3-glucanase was a more significant factor than chitinase for determining the mycolytic potential of bacteria and their ability to utilize the mycelium of phytopathogenic fungi as a growth substrate.

Key words: mycolytic activity, microbial antagonism, chitinase, β-1,3-glucanase, *Bacillus subtilis, Paenibacillus ehimensis, Bipolaris sorokiniana.*

DOI: 10.1134/S0026261708060088

Mycolytic activity is usually considered a form of microbial antagonism, which ensures the capability of various microorganisms for the lysis of fungal hyphae [1, 2]. This property is typical of many groups of soil bacteria and can serve as a criterion for the selection of antagonistic strains to develop effective biological means for controlling pathogenic micromycetes [3–7]. The local lysis, or complete degradation of fungal hyphae by bacteria, is a complicated process of microbial interactions governed by competition for nutrients. The mycolytic activity of bacteria can be due to the production of antibiotics, which specifically inhibit the biosynthesis of fungal cell walls (CW) [8]; to the secretion of hydrolytic enzymes degrading the CW components [9–11]; or to the synthesis of amphiphilic peptides and other surface-active compounds, impairing permeability of fungal cytoplasmic membranes [12]. The autolysis of fungi can also be caused by the exhaustion of endogenous nutrients in the course of their prolonged interactions with bacteria growing at the expense of the nutrients excreted by the fungi [13]. These processes can be accompanied by bacterial growth and active colonization of both mature and growing fungal hyphae [14]. In the studies of bacterial lytic factors and the application of mycolytic bacteria for biocontrol of pathogenic fungi, much attention has been devoted to extracellular enzymes degrading fungal cell walls [6, 15, 16]. The main feature of microbial mycolytic systems is considered to be the presence of chitinases capable of hydrolyzing chitin, a structural polysaccharide of fungal cell walls determining their rigidity [17]. In most cases, chitinases are inducible enzymes, which are produced in the presence of specific substrates. The efficacy of inducing chitinase synthesis in bacteria can depend considerably on the origin of the chitin. However, chitinolytic activity is usually considered among the most important criteria determining bacterial antagonistic properties, which correlates with the mycolytic activity of bacteria and their ability to utilize fungal biomass [6, 18]. At the same time, the antifungal functions of chitinases remain unclear because of the contradictory results of their testing in vitro. Therefore, of great importance is detailed study of the functional variety of chitinases produced by representatives of closely related and distant taxonomic groups of bacteria based on the assessment of their mycolytic properties. Aerobic endospore-forming bacteria can be used as type objects for such studies since they possess both antagonistic properties and the ability to synthesize chitinolytic enzymes.

In this study, we assessed the mycolytic activity of 25 strains of *Bacillus subtilis* and *Paenibacillus ehimensis* capable of induced synthesis of chitinolytic enzymes. The aim of this work was to investigate a cor-

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

relation between chitinase production and the mycolytic activity of antagonistic bacilli, and to characterize specific factors determining the mycolytic properties of the representatives of different taxonomic groups of aerobic spore-forming bacteria.

MATERIALS AND METHODS

The study was carried out with 27 chitinolytic strains of antagonistic bacilli isolated at the Institute of Biology, Ufa Research Center, Russian Academy of Sciences [19]. Most members (18 strains) of this group were identified, using classical manuals [20, 21], as *Bacillus subtilis*, whereas the other strains were assigned to the species *Paenibacillus ehimensis* on the basis of their phenotypic properties and the results of the sequence analysis of the 16S rRNA gene [22, 23]. In comparative studies of mycolytic activities of different strains, the antagonistic strain *B. subtilis* IB-54 unable to synthesize extracellular chitinases was used as the typical control strain. Bacilli were maintained on a solid medium containing 0.2% (by weight) of colloidal chitin from crab shells [24].

The cereal root rot pathogen *Bipolaris sorokiniana* (Sacc.) Shoemaker (= *Cohliobolus sativus* (S. Itod Kurib.) Drechler ex Dastur) from the collection of the Institute of Biology, Ufa Research Center, Russian Academy of Sciences [25],was used as the subject for the study of the mycolytic properties of antagonistic bacilli. The fungus was maintained on a Czapek medium and on potato–glucose agar (PGA) and was transferred onto a fresh medium every 2 months. The mycolytic activity of bacilli was assayed with living mycelium under mixed cultivation; the capacity for growth in the medium containing the autoclaved *B. sorokiniana* mycelium as the sole source of carbon and nitrogen was also determined. The ability of extracellular enzymes produced by antagonistic bacilli to hydrolyze the cell walls of *B. sorokiniana* was also determined [24].

Mixed cultivation of bacilli and *B. sorokiniana* was carried out in liquid Czapek medium under static conditions at 28°C for 7 days. Bacilli were preliminary grown for 18 h on a UVMT-250-12 shaker (170 rpm) at 36.5 ± 0.5°C in a medium containing the following (g/l): potato starch, 10.0; peptone, 3.0; yeast extract, 2.0; corn extract, 1.0; $(NH_4)_2HPO_4$, 1.0; KH_2PO_4, 1.0; $MgSO_4 \cdot 7H_2O$, 0.3; $CaCl_2 \cdot 2H_2O$, 0.15. The cells were washed, suspended in sterile NaCl solution (0.85%), and introduced into the Czapek medium (at least 10^5 cells/ml) simultaneously with the fungus (mixed synchronous culture), or 24 h after fungus inoculation (mixed postculture). The inoculum of *B. sorokiniana* was prepared by washing off a 10-day culture of the fungus (at least 10^3 conidia/ml). The effect of bacilli on fungal growth was examined visually by light microscopy. The degree of inhibition of fungal growth was expressed as the percentage difference between the dry weight of mycelium grown in the control (taken as 100%) and that in the experimental variants [24]. The mycelium was washed with distilled water on weighed Whatman 1 filters and then dried at 105°C to attain a constant dry weight. In some cases, the titer of bacterial cells in the mixed culture was determined by plating serial dilutions of the culture on nutrient agar.

In order to use *B. sorokiniana* mycelium as a growth substrate, the fungus was cultivated in the liquid potato medium supplemented with 1% (wt/vol) of sucrose at 28°C for 2 weeks. The mycelium was harvested by filtration, washed sequentially with tap water and distilled water, air-dried, disintegrated in a EM-3A laboratory mill, and added into the liquid medium (0.5% by weight) as the sole source of carbon and nitrogen. The medium was prepared by using tap water without the addition of mineral salts and sterilized at 121°C for 30 min. The same mycelium, which was additionally washed and dried after disintegration, was used as a substrate for the assessment of the hydrolytic activity of bacterial enzymes towards the cell walls of *B. sorokiniana* [24]. The growth of bacilli in the mycelium-containing medium was followed by measurement of the optical density of the culture on an SF-46 spectrophotometer at 630 nm.

In comparative studies of the production of chitinolytic enzymes, bacilli were grown in a liquid medium containing colloidal chitin from crab shells and biomass of the basidiomycete *Macrolepiota procera* (Fr.) Sing. The activities of chitinase and other hydrolases, such as β-1,3-glucanase, chitosanase, and protease, were measured. The activities of glycosyl hydrolases were assessed by the rate of formation of reducing sugars, which were released during hydrolysis of colloidal chitin, colloidal chitosan, and laminarin [24]. One unit of activity was defined as the amount of enzyme that catalyzed the formation of 1 μM-equiv of the corresponding monomer per 1 min in 1 ml of the reaction mixture [24]. The protease activity was expressed in units of commercial proteinase K (Sigma, United States) and calculated using the calibration curve obtained with azocasein (Sigma, United States) as the substrate [24].

The experiments were conducted in triplicate; statistical data processing was performed with the Excel-2000 program. The empirical correlation coefficient was calculated by the formula $r_{x,y} = \left[1/n \sum_{i=1}^{n} (x_i - x_{cp})(y_i - y_{cp})\right] / S_x S_y$. Taking into account scant sampling, ($n < 30$), the correction factor was $[1 + (1 - r^2)/(2(n - 3))]$. The correlation coefficient was determined for a 5% level of significance ($\alpha = 5\%$).

RESULTS AND DISCUSSION

Under synchronous mixed cultivation of bacteria and *B. sorokiniana* in liquid Czapek medium, most bacterial cultures (20 out of 27 strains) completely inhib-

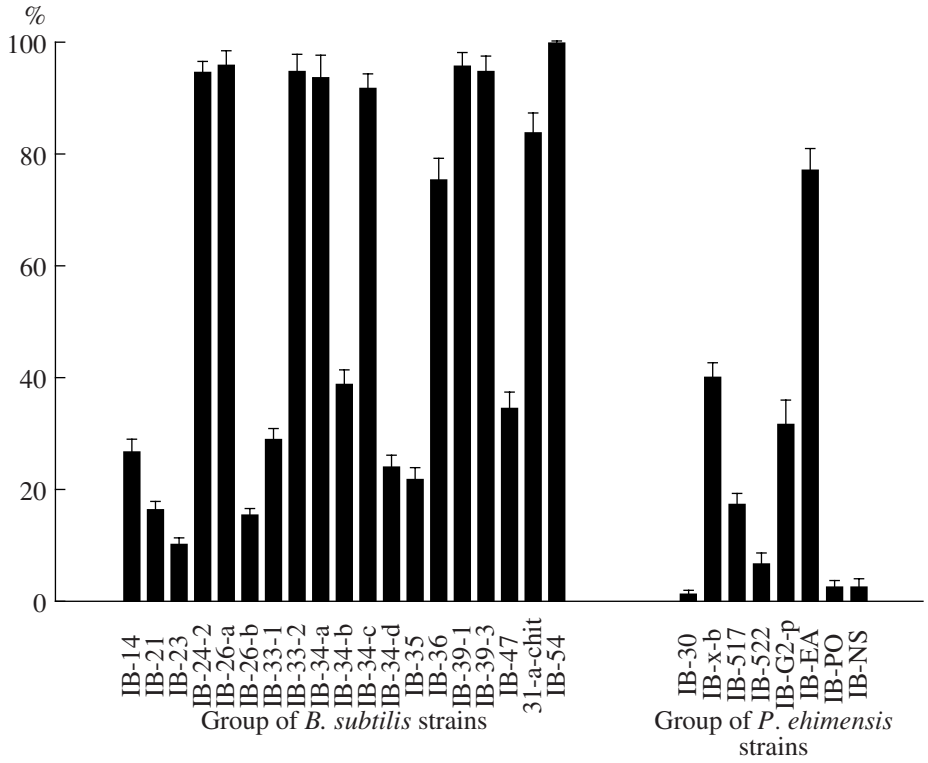

Fig. 1. Inhibition of *B. sorokiniana* growth by chitinolytic strains (except for *B. subtilis* IB-54) belonging to different groups of bacilli under their postinoculation into Czapek medium containing a 24-h fungal culture. The results were obtained on the seventh day of mixed cultivation. The ordinate shows the level of fungal growth inhibition, % of the control.

ited (by 100%) conidium germination and subsequent mycelium development. The postinoculation of bacteria into the medium containing a 24-h fungal culture reduced antagonistic action (by 60–90%) in half of the *B. subtilis* strains studied, whereas other strains inhibited fungal growth to a high extent (Fig. 1). Under these conditions, the group of *P. ehimensis* strains generally exhibited a lower antagonistic activity than the *B. subtilis* strains (Fig. 1), which coincided with different growth intensities in these bacterial groups. Visual observations revealed certain patterns in the action of the representatives of both bacterial groups toward a 24-hour fungal culture. Most strains of *B. subtilis* evoked segmental swelling of fungal hyphae, with the subsequent formation of spheroplast-like structures. *B. subtilis* strains differed only in the number of such structures, their distribution, and localization on the mycelium (Figs. 2a–2e). In many cases, further incubation resulted in rapid or gradual lysis of spherical structures (Figs. 2f, 2g). Only a few strains of *B. subtilis* caused abnormal germination of *B. sorokiniana* conidia, or pronounced degradation and lysis of hyphae and conidia (Figs. 2h, 2i). Strains of *P. ehimensis* exhibited mainly the lytic action or caused the abnormal development of mycelium and its dystrophic degeneration (Figs. 2j, 2k), although some strains, like *B. subtilis*, promoted the formation of spheroplast-like structures on large segments of *B. sorokiniana* hyphae (Fig. 2l).

According to the literature data, the swelling of fungal hyphae and the formation of spherical structures is a typical result of the action of many antagonistic bacteria toward various micromycete species [26–28]. This visual similarity in the bacterial action is possibly due to a common mechanism of action in the compounds produced by antagonists, as well as to the structural similarity of the cell walls and CPM of fungi [8, 17]. It should be noted that chitinolytic activity of all the studied strains cultivated in the Czapek medium without a specific substrate was negligible or absent (Table 1). It is obvious that the observed abnormal development of *B. sorokiniana*, such as mycelium swelling and the formation of spherical structures on the hyphae in the presence of *B. subtilis* strains (Figs. 2b–2e), cannot be explained by the production of bacterial lytic enzymes similar to chitinase. The role of chitinases and other enzymes in the mycolytic action of some representatives of the *P. ehimensis* group cultivated together with fungi in the Czapek medium (Fig. 2n) needs further investigation. It is not improbable that the degradation of hyphae and spores of *B. sorokiniana* can be caused by the local secretion of trace amounts of lytic enzymes

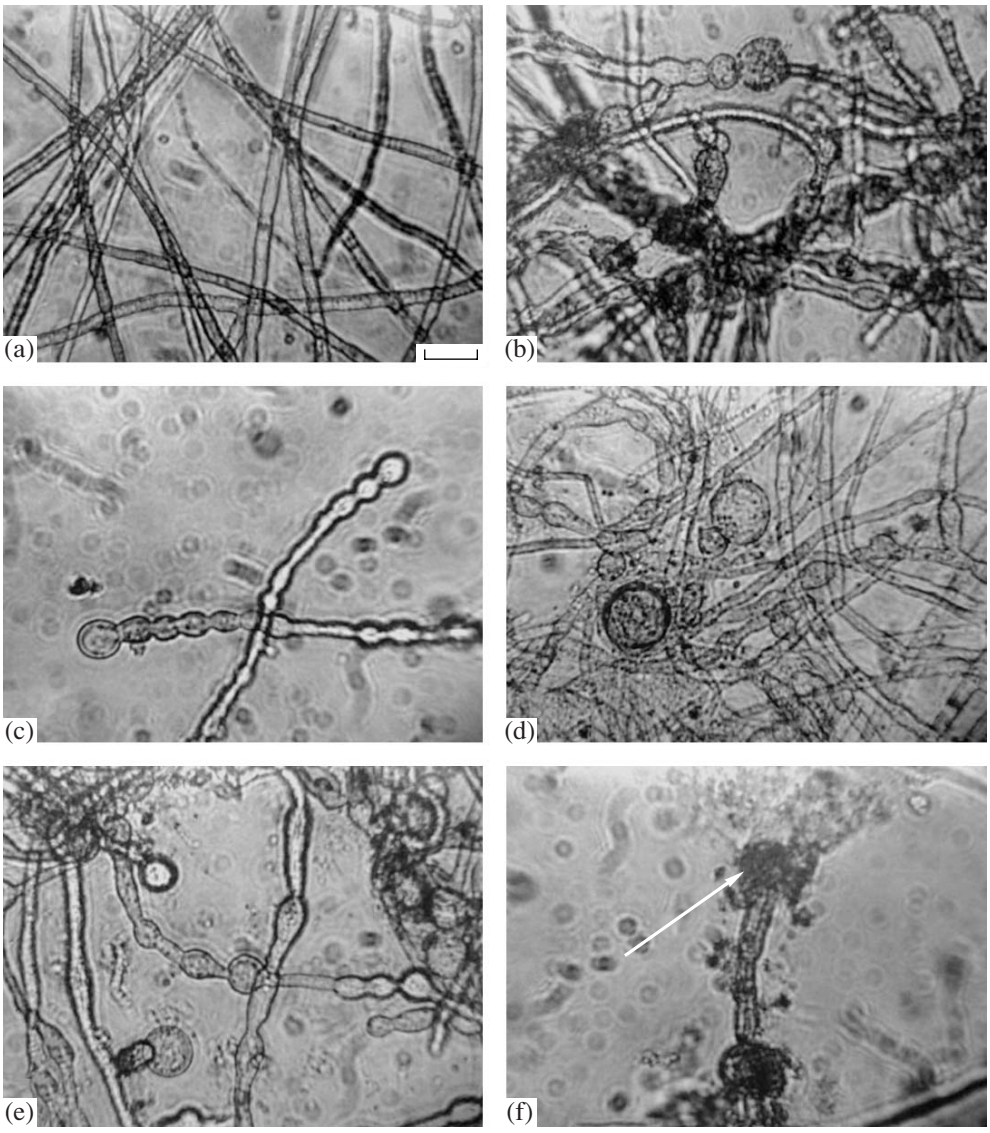

Fig. 2. The effect of chitinolytic strains of *B. subtilis* (b–l) and *P. ehimensis* (m–h) on the development and hypha morphology of a 24-hour culture of *Bipolaris sorokiniana* grown under mixed incubation in Czapek medium for 1–4 days at 28°C. Control (a 4-day culture of *B. sorokiniana*) (a); (b–j), formation of spheroplast-like structures (b–e) on fungal hyphae and their lysis (f, j, indicated with arrows) caused by *B. subtilis* strains IB-21, IB-24-2, IB-34-a, IB-26-a, IB-36, and IB-33-1, respectively; (k, l), impairment of conidium germination and partial lysis of hyphae and conidia by *B. subtilis* strains IB-36 and IB-47; (m–h), the changes in fungal morphology caused by *P. ehimensis* strains IB-G2-p, IB-X-b, and IB-30. The scale bar represents 120 μm.

on their surfaces during the contact interactions between the fungus and bacteria.

The strains of both groups of aerobic spore-forming bacteria differed not only in their mycolytic activities toward the living culture of *B. sorokiniana*, but also in their ability to utilize the autoclaved mycelium of this fungus as the sole source of carbon and nitrogen, which reflected their capacity for the production of enzymes that hydrolyze the polymers of fungal cell walls. Most of the *B. subtilis* strains were characterized by poor growth or did not actually grow in a liquid medium containing *B. sorokiniana* biomass, unlike strains of *P. ehimensis* (except for the strain IB-31-c-chit) (Table 2). The growth parameters of *P. ehimensis* strains correlated well with the levels of their chitinolytic activity (Table 2) and the excretion of other hydrolytic enzymes, such as β-1,3-glucanase and protease (Table 3). The group of *P. ehimensis* strains was characterized by a high production of proteases, and glucanases. These results indicate the considerable role of

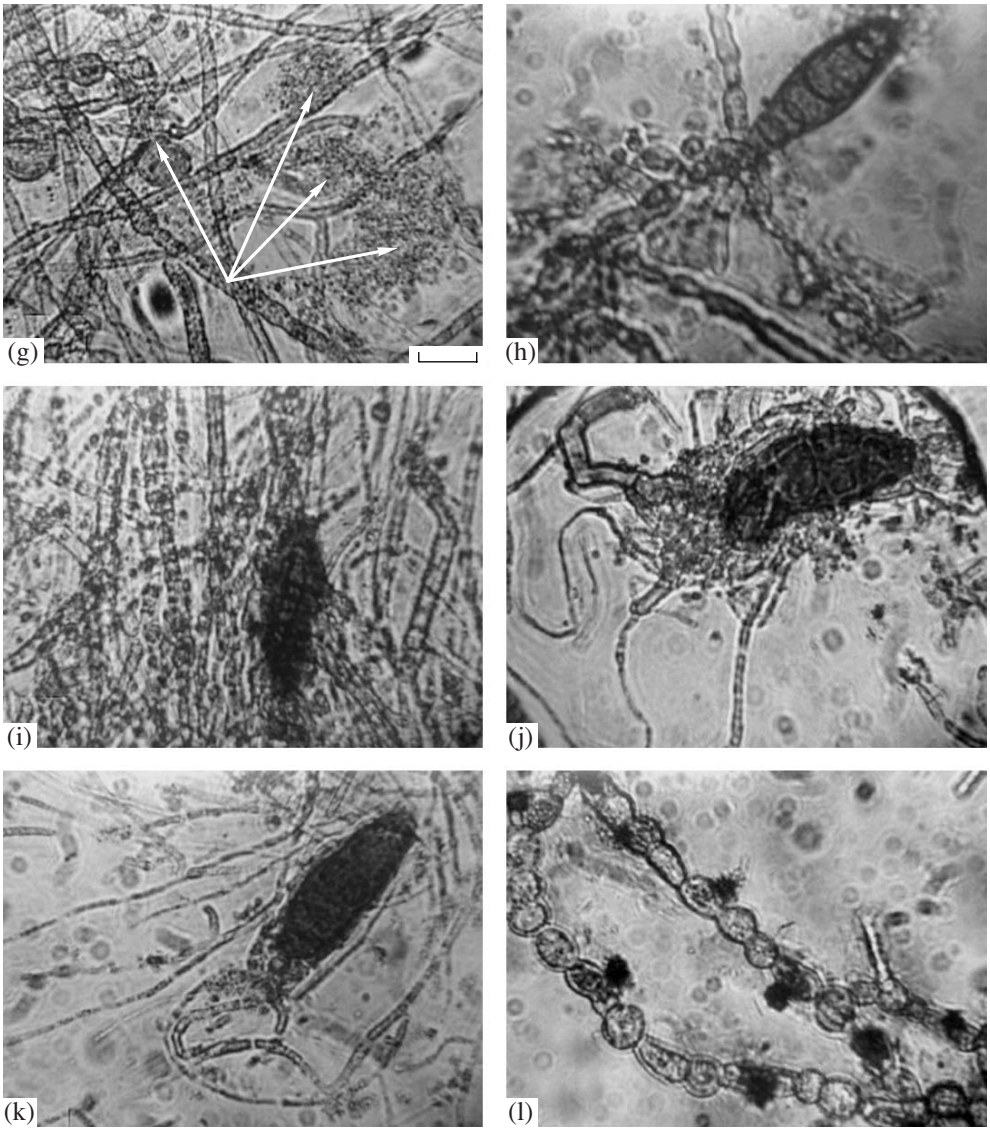

Fig. 2. Contd.

enzymes of the mycolytic complex in the degradation of *B. sorokiniana* mycelium by *P. ehimensis*, which provided bacteria with available nutrients.

It should be noted that the parameters of synthesis and excretion of lytic enzymes differed when the strains studied were grown in the media containing other chitin-containing substrates, organic nitrogen sources, and growth factors. In the medium containing 0.2–0.4% (by weight) of colloidal chitin from crab shells and the biomass of the higher fungus *Macrolepiota procera* (≤0.4 wt %, most representatives of *B. subtilis* exhibited considerably higher chitinase activity than *P. ehimensis* strains; the level of chitinase activity was rather high in the strains of both groups (Fig. 3a). These results contradict the data presented in Table 2, which indicate a low capacity of *B. subtilis* strains for utilization of *B. sorokiniana* mycelium as the growth substrate. Thus, the synthesis of extracellular chitinases in bacilli of different genera was induced by various substrates with different physicochemical properties, such as chitins obtained from fungi or from crab shells. In this experimental variant, the mycolytic properties of bacteria can be also characterized by the activities of extracellular glycosyl hydrolases degrading the cell walls of *B. sorokiniana*. No correlation between the level of glycosyl hydrolases (Fig. 3b) and chitinolytic activity of the strains (Fig. 3a) was revealed ($R_{x,y}$ = –0.014). However, a correlation was shown between the mycolytic activity of extracellular enzymes (Fig. 3b) and the ability of *P. ehimensis* strains to grow

Table 1. Chitinolytic activity (U/ml) of *B. subtilis* and *P. ehimensis* strains grown in monoculture (1) and under mixed cultivation with *B. sorokiniana* (2) in liquid Czapek medium for 6 days

Group of *B. subtilis* strains	1	2	Group of *B. subtilis* strains	1	2	Group of *P. ehimensis* strains	1	2
IB-14	NA	NA	IB-34-b	NA	NA	IB-30	NA	NA
IB-21	<0.001	NA	IB-34-c	NA	NA	IB-X-b	<0.001	0.002
IB-23	NA	NA	IB-34-d	NA	NA	IB-517	NA	0.001
IB-24-2	NA	NA	IB-35	NA	NA	IB-522	NA	<0.001
IB-26-a	NA	NA	IB-36	NA	NA	IB-G2-p	0.001	0.002
IB-26-b	NA	NA	IB-39-1	NA	NA	IB-EA-b	0.001	0.001
IB-33-1	<0.001	0.001	IB-39-3	NA	NA	IB-PO	NA	0.001
IB-33-2	NA	<0.002	IB-47	NA	NA	IB-31-c-chit	ND	ND
IB-34-a	NA	<0.001	IB-31-a-chit	NA	NA	IB-NS	NA	NA

Note: NA stands for "no activity"; ND means "no data."

Table 2. Biomass growth and secretion of chitinolytic enzymes by strains belonging to different taxonomic groups of bacilli cultivated in liquid medium containing 0.5% of *B. sorokiniana* mycelium as the sole source of carbon and nitrogen on a shaker (170 rpm) at 36.5°C for 72 h

Strain	OD_{630}	Chitinase activity, U/ml	Strain	OD_{630}	Chitinase activity, U/ml
Strains of high productivity					
P. ehimensis IB-30	1.39 ± 0.09	0.12 ± 0.007	*P. ehimensis* IB-522	1.11 ± 0.10	0.08 ± 0.012
P. ehimensis IB-X-b	1.19 ± 0.08	0.07 ± 0.008	*P. ehimensis* IB-G2-p	0.97 ± 0.02	0.10 ± 0.007
P. ehimensis IB-517	1.07 ± 0.05	0.08 ± 0.004	*P. ehimensis* IB-EA(b)	0.83 ± 0.02	0.04 ± 0.006
Strains of medium productivity					
B. subtilis IB-14	0.37 ± 0.02	0	*B. subtilis* IB-34-c	0.23 ± 0.01	0.01 ± 0.003
B. subtilis IB-21	0.76 ± 0.02	0.01 ± 0.003	*B. subtilis* IB-34-d	0.17 ± 0.01	~0.005
B. subtilis IB-24-2	0.22 ± 0.01	< 0.001	*B. subtilis* IB-31-a-chit	0.35 ± 0.01	0
B. subtilis IB-26-a	0.30 ± 0.01	0	*P. ehimensis* IB-PO	0.54 ± 0.02	0.04 ± 0.007
B. subtilis IB-33-1	0.29 ± 0.01	0	*P. ehimensis* IB-NS	0.75 ± 0.03	0.06 ± 0.004
B. subtilis IB-33-2	0.26 ± 0.01	0.01 ± 0.002			
Strains of low productivity					
B. subtilis IB-23	0.06 ± 0.01	0.02 ± 0.003	*B. subtilis* IB-36	0.04 ± 4.10^{-3}	0
B. subtilis IB-26-b	$0.02 \pm 3 \cdot 10^{-3}$	0	*B. subtilis* IB-39-1	0.03 ± 4.10^{-3}	<0.001
B. subtilis IB-34-a	0.13 ± 0.01	~0.004	*B. subtilis* IB-39-3	0.05 ± 0.01	0
B. subtilis IB-34-b	0.09 ± 0.01	~0.001	*B. subtilis* IB-47	0.03 ± 4.10^{-3}	<0.001
B. subtilis IB-35	0.09 ± 0.01	0.01 ± 0.002	*P. ehimensis* IB-31-c-chit	0.02 ± 4.10^{-3}	~0.001

Note: OD_{630} means the optical density of the culture suspension at 630 nm.

Table 3. Production (U/ml) of β-1,3-glucanase and protease by strains belonging to two taxonomic groups of bacilli grown in liquid medium containing 0.5% of *B. sorokiniana* mycelium as the sole source of carbon and nitrogen on a shaker (170 rpm) at 36.5°C for 72 h

Group of *B. subtilis* strains

Strain	β-1,3 glucanase activity, U ml^{-1}	Protease activity, U ml^{-1}	Strain	β-1,3 glucanase activity, U ml^{-1}	Protease activity, U ml^{-1}
IB-14	0.01 ± 0.01	0.18 ± 0.02	IB-34-b	0	0.07 ± 0.01
IB-21	0	0.55 ± 0.06	IB-34-c	0	0.21 ± 0.02
IB-23	0	0.05 ± 0.006	IB-34-d	0	0.18 ± 0.02
IB-24-2	0	0.14 ± 0.02	IB-35	0	0.17 ± 0.02
IB-26-a	0.005 ± 0.002	0.19 ± 0.02	IB-36	0.007 ± 0.003	0.09 ± 0.01
IB-26-b	0	0.02 ± 0.006	IB-39-1	0.02 ± 0.004	0.04 ± 0.005
IB-33-1	0	0.35 ± 0.04	IB-39-3	0.03 ± 0.006	0.08 ± 0.01
IB-33-2	0.02 ± 0.002	0.31 ± 0.04	IB-47	<0.01	0.07 ± 0.01
IB-34-a	0.02 ± 0.003	0.17 ± 0.03	IB-31-a-chit	<0.01	0.31 ± 0.03

Group of *P. ehimensis* strains

IB-30	1.48 ± 0.11	1.03 ± 0.08	IB-EA-b	0.12 ± 0.01	0.67 ± 0.07
IB-X-b	0.76 ± 0.04	1.25 ± 0.15	IB-PO	0.29 ± 0.04	0.52 ± 0.06
IB-517	1.56 ± 0.11	0.80 ± 0.09	IB-31-c-chit	0.01 ± 0.004	<0.02
IB-522	0.51 ± 0.05	0.75 ± 0.09	IB-NS	0.52 ± 0.04	0.84 ± 0.08
IB-G2-p	1.37 ± 0.10	0.91 ± 0.09			

actively in the medium with 0.5% of *B. sorokiniana* biomass and to produce β-1,3-glucanase (Tables 2–4). The cell wall polymers of the fungi *B. sorokiniana* and *M. procera* differed in their capability for the induction of glucanase synthesis. Unlike *B. subtilis*, strains of *P. ehimensis* also secreted chitosan-degrading hydrolases, whereas the levels of protease activity in both bacterial groups were similar (Table 4). These results indicate that mycolytic activity of bacteria belonging to different genera implies the synthesis of different hydrolytic enzymes involved in the degradation of fungal cell walls. It was demonstrated with strains of *B. subtilis* that production of extracellular chitinases cannot be considered as the sole or a key factor in determining the mycolytic properties of bacilli. Bacteria of different taxonomic groups probably possess different mechanisms for mycolytic activity, and the activities of individual strains may vary considerably depending on the fungal species used as growth substrates.

Thus, based on the differences in the hydrolytic activities of two distant taxonomic groups of aerobic spore-forming bacteria, we showed that chitinolytic activity was not the main criterion determining the mycolytic properties of bacilli. Comparative studies of bacterial extracellular enzymes demonstrated that the effect of bacilli on *B. sorokiniana* cell walls depended on the spectrum of hydrolyses secreted by bacteria under different conditions. It was shown that the presence of β-1,3-glucanase was a more important criterion than that of chitinase for screening the myc-

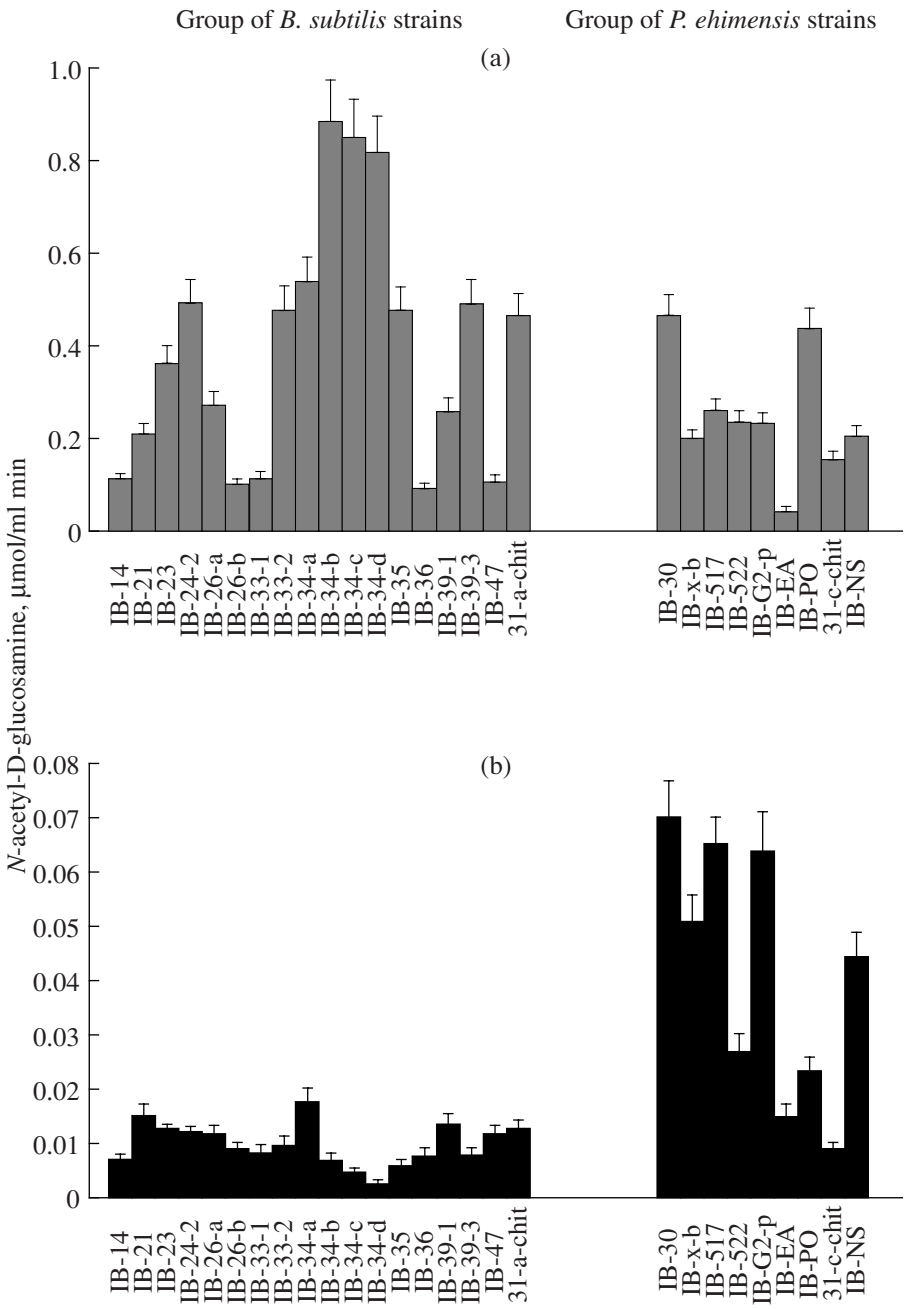

Fig. 3. Chitinase (a) and mycolytic (b) activities of different strains of antagonistic bacilli grown in the medium containing 0.2% of colloidal chitin from crab shells and 0.4% of the biomass of the fungus *Macrolepiota procera* (Fr.) Sing. on a shaker (170 rpm) at 36.5°C for 72 h. The ordinate on the upper diagram (a) shows the rate of *N*-acetyl-D-glucosamine formation (μmol/ml min) from hydrolysis of 1% suspension of colloidal chitin. The ordinate on the lower diagram (b) indicates the rate of the reducing sugar release (μmol/ml min) under the action of bacterial enzymes on a 1% suspension of *Bipolaris sorokiniana* disintegrated cell walls. *N*-acetyl-D-glucosamine was used as a standard.

olytic strains of antagonistic bacteria. The mycolytic activity of bacteria in vitro and in vivo resulting from the synthesis of various extracellular hydrolases can depend on environmental factors to a greater extent than other forms of microbial antagonism. This finding should be taken into account in the development of modern means for biological control of phytopathogenic fungi.

Table 4. Production (U/ml) of chitosanase, β-1,3-glucanase, and protease by strains belonging to two taxonomic groups of bacilli grown in liquid medium containing 0.2% of colloid chitin from crab shells and 0.4% of the biomass of the fungus *Macrolepiota procera* (Fr.), Sing

Group of *B. subtilis* strains

Strain	Chitosanase activity, U ml^{-1}	β-1,3 glucanase activity, U ml^{-1}	Protease activity, U ml^{-1}	Strain	Chitosanase activity, U ml^{-1}	β-1,3 glucanase activity, U ml^{-1}	Protease activity, U ml^{-1}
IB-14	0	0	1.53 ± 0.14	IB-34-c	0	0	0.47 ± 0.05
IB-21	0	0	1.61 ± 0.17	IB-34-d	0	0	0.48 ± 0.05
IB-23	0	0	1.96 ± 0.20	IB-35	0	0	1.67 ± 0.18
IB-24-2	0.01	0	1.12 ± 0.10	IB-36	0	0.08	1.79 ± 0.19
IB-26-a	0.01	0.01	1.56 ± 0.16	IB-39-1	0.01	0	1.35 ± 0.14
IB-26-b	0	0	1.60 ± 0.17	IB-39-3	0	0	0.48 ± 0.05
IB-33-1	0	0	1.50 ± 0.16	IB-47	0	0.03	1.78 ± 0.18
IB-33-2	0.01	0	1.79 ± 0.20	IB-31-a-x	0	0	1.55 ± 0.16
IB-34-a	0.03	0	1.37 ± 0.14	IB-54*	0	0	1.67 ± 0.18
IB-34-b	0	0	0.34 ± 0.04				

Group of *P. ehimensis* strains

Strain	Chitosanase activity, U ml^{-1}	β-1,3 glucanase activity, U ml^{-1}	Protease activity, U ml^{-1}	Strain	Chitosanase activity, U ml^{-1}	β-1,3 glucanase activity, U ml^{-1}	Protease activity, U ml^{-1}
IB-30	0.22 ± 0.02	7.28 ± 0.75	1.29 ± 0.14	IB-EA-b	0	0.18 ± 0.02	1.64 ± 0.17
IB-X-b	0.36 ± 0.04	5.94 ± 0.65	1.65 ± 0.17	IB-PO	0.27 ± 0.03	0.30 ± 0.03	0.42 ± 0.05
IB-517	0.34 ± 0.04	6.77 ± 0.70	1.24 ± 0.13	IB-31-x	0	0	0.19 ± 0.02
IB-522	1.06 ± 0.11	0.36 ± 0.05	0.95 ± 0.10	IB-NS	0.17 ± 0.02	2.61 ± 0.30	1.54 ± 0.16
IB-G2-p	0.49 ± 0.05	6.00 ± 0.65	1.06 ± 0.12				

Note: * Strain IB-54 is a type strain of antagonistic bacilli exhibiting no chitinolytic activity.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project 07-04-00812-a).

REFERENCES

1. Khudyakov, Ya.P., Lytic Effect of Soil Bacteria on Soil Fungi, *Mikrobiologiya*, 1935, vol. 4, no. 2, pp. 193–203.
2. Mitchell, R. and Alexander, M., Lysis of Soil Fungi by Bacteria, *Can. J. Microbiol.*, 1969, vol. 15, no. 9, pp. 169–177.
3. Mitchell, R. and Alexander, M., Mycolytic Phenomenon and Biological Control of *Fusarium* in Soil, *Nature*, 1961, pp. 109–110.
4. Davies, D.A. and Pope, A.M., Mycolase, a New Kind of Systemic Antimycotic, *Nature*, 1978, vol. 273, pp. 235–236.
5. Podile, A.R. and Prakash, A.P., Lysis and Biological Control of *Aspergillus niger* by *Bacillus subtilis* AF1, *Can. J. Microbiol.,* 1996, vol. 42, no. 6, pp. 533–538.
6. Patil, R.S., Ghormade, V., and Deshpande, M.V., Chitinolytic Enzymes: An Exploration, *Enz. Microb.Technol.*, 2000, vol. 26, pp. 473–483.
7. Ryazanova, L.P., Stepnaya, O.A., Suzina, N.E., and Kulaev, I.S., Antifungal Action of the Lytic Enzyme Complex from *Lysobacter* sp. XL1, *Proc. Biochem. Soc.*, 2005, vol. 40, pp. 557–564.
8. Debono, M. and Gordee, R.S., Antibiotics That Inhibit Fungal Cell Wall Development, *Annu. Rev. Microbiol.*, 1994, vol. 48, pp. 471–497.
9. Horikoshi, K. and Iida, S., Lysis of Fungal Mycelia by Bacterial Enzymes, *Nature*, 1958, vol. 181, no. 4613, pp. 917–918.
10. Skujins, J.J., Potgieter, H.J., and Alexander, M., Dissolution of Fungal Cell Walls by a Streptomycete Chitinase and Beta-(1,3)-Glucanase, *Arch. Biochem. Biophys.*, 1965, vol. 111, no. 2, pp. 358–364.
11. Sivan, A. and Chet, I., Degradation of Fungal Cell Walls by Lytic Enzymes of *Trichoderma harzianum, J. Gen. Microbiol.,* 1989, vol. 135, pp. 675–682.
12. Maget, D.R. and Peypoux, F., Iturins, a Special Class of Pore-Forming Lipopeptides: Biological and Physicochemical Properties, *Toxicol*, 1994, vol. 87, pp. 151–174.
13. Nikonorova, A.K., Interaction Patterns of *Bacillus subtilis* and *Helminthosporium sativum* Pam., King et Vakke, *Mikol. Fitopatol.*, 1996, vol. 30, no. 1, pp. 69–73.
14. Voznyakovskaya, Yu.M. and Trufanova, A.K., Interaction of *Helminthosporium sativum*, a Wheat Root Rot Pathogen, with Saprophytic Soil Bacteria, *Mikol. Fitopatol.*, 1988, vol. 22, no. 2, pp. 157–161.
15. Budi, S.W., Tuinen Van, D., Arnold, C., Dumas-Gaudot, E., Gianinazzi-Pearson, V., and Gianinazzi, S. Hydrolytic Enzyme Activity of *Paenibacillus* sp. Strain B2 and Effects of the Antagonistic Bacterium on Cell Integrity of Two Soil-Borne Pathogenic Fungi, *Appl. Soil. Ecol.*, 2000, vol. 15, pp. 191–199.
16. Viterbo, A., Ramot, O., Chernin, L., and Chet, I., Significance of Lytic Enzymes from *Trichoderma* spp. in the Biocontrol of Fungal Plant Pathogens, *Antonie van Leeuwenhoek Microbiol. Serol.*, 2002, vol. 81, pp. 549–556.
17. Odds, F.C., Brown, A.J.P., and Gow, N.A.R., Antifungal Agents: Mechanisms of Action. Review, *Trends in Microbiology*, 2003, vol. 11, no. 6, pp. 272–279.
18. Gohel, V., Singh, A., Vimal, M., Ashwini, P., and Chhatpar, H.S., Bioprospecting and Antifungal Potential of Chitinolytic Microorganisms. Review, *Afr. J. Biotechnol.*, 2006, vol. 5, pp. 54–72.
19. Melent'ev, A.I. and Erkeev, A.M., Investigation of Antagonism between Soil Bacilli and *Fusarium* Lk:Fr Micromycetes, *Mikrobiol. Zhurn*, 1990, vol. 52, no. 1, pp. 53–56.
20. Gordon, R.E., Haynes, W.C., and Pang, C.H., The Genus *Bacillus, Agricultural Handbook N 427*, The US Department of Agriculture, Washington, DC, 1973.
21. *Bergey's Manual of Systematic Bacteriology*, Baltimore, Williams & Wilkins, 1986.
22. Kuroshima, K.I., Sakane, T., Takata, R., and Yokota, A., *Bacillus ehimensis* sp. nov. and *Bacillus chitinolyticus* sp. nov., New Chitinolytic Members of the Genus *Bacillus, Int. J. Syst. Bacteriol.*, 1996, vol. 46, pp. 76–80.
23. Lee, J.-S., Pyun, Y.-R., and Bae, K.S., Transfer of *Bacillus ehimensis* and *Bacillus chitinolyticus* to the Genus *Paenibacillus* with Emended Descriptions of *Paenibacillus ehimensis* comb. nov. and *Paenibacillus chitinolytic* comb. nov., *Int. J. Syst. Evol. Microbiol.*, 2004, vol. 54, pp. 929–933.
24. Aktuganov, G.E., Galimzyanova, N.F., Melent'ev, A.I., and Kuz'mina, L.Yu., Extracellular Hydrolases of Strain *Bacillus* sp. 739 and Their Involvement in the Lysis of Micromycete Cell Walls, *Mikrobiologiya*, 2007, vol. 76, no. 4, pp. 471–479 [*Microbiology* (Engl. Transl.), vol. 76, no. 4, pp. 413–420].
25. Bilai, V.I., Gvosdek, R.I., Skripal', I.G., Kraev, V.G., Ellanskya, M.A., Zirka, G.I., and Mudras, V.A., *Microorganizmy—vozbuditeli boleznei rastenii* (Microorganisms as Causal Agents of Plant Diseases), Kiev: Naukova Dumka, 1988.
26. Dass, C. and Teyegaga, A., Growth Suppression of Some Wood-Decay Fungi and Other Fungi by *Bacillus subtilis, Aust. J. Bot.*, 1996, vol. 44, pp. 705–712.
27. San-Lang, W., Yieh, T.-C., and Shih, I.-L., Purification and Characterization of a New Antifungal Compound Produced by *Pseudomonas aeruginosa* K-187 in a Shrimp and Crab Shell Powder Medium, *Enz. Microb. Technol.*, 1999, vol. 25, pp. 439–446.
28. Jung, W.J., An, K.N., Jin Y.L., Park, R.D., Lim, K.T., Kim, K.Y., and Kim, T.N., Biological Control of Damping-off Caused by *Rhizoctonia solani* Using Cjitinase-Producing *Paenibacillus illinoisensis* KJA-424, *Soil Biol. Biochem.,* 2003, vol. 35, pp. 1261–1264.