ISSN 0026-2617, Microbiology, 2011, Vol. 80, No. 2, pp. 205–215. © Pleiades Publishing, Ltd., 2011.
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EXPERIMENTAL ARTICLES

Specificity of the Chitinolytic Microbial Complex of Soils Incubated at Different Temperatures

N. A. Manucharova[a, 1], A. N. Vlasenko[a], E. V. Men'ko[b], and D. G. Zvyagintsev[a]

[a] *Department of Soil Biology, Faculty of Soil Science, Moscow State University, Moscow, 119992 Russia*
[b] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*

Received February 2, 2010

Abstract—The structural and functional specificity of the chitinolytic microbial complex changes dramatically depending on the incubation temperature of soil microcosms. It was shown that the highest rates of chitin degradation occurred in desert soils at high temperatures (50°C); in the moderate and northern zones, these rates peaked at lower temperatures (5°C). The role of prokaryotes as the main chitin degraders in soils incubated at high temperatures, with fungi more actively participating in chitin decomposition at low temperatures, was shown for the first time. Fluorescent in situ hybridization (FISH) revealed the predominance of actinomycetes in the metabolically active chitinolytic prokaryotic complex of desert soils (high temperatures); in the soils of the northern latitudes (low temperatures), proteobacteria prevailed. The relationship between the taxonomic position of the dominant members of the chitinolytic complex of soil microorganisms, isolated in pure cultures with the dominant phylogenetic groups and the sequence types obtained by using molecular biological techniques (FISH) was revealed.

Keywords: chitinolytic microorganisms, thermophily, biopolymer transformation, physiologically active cells (FISH method), ecology.

DOI: 10.1134/S002626171102010X

Temperature is among the factors influencing microbial growth and activity in a system. On the one hand, the influence of psychrophilic and psychrotrophic microorganisms on natural processes occurring in the cold and temperate climate zones typical of the greater part of Russia is undeniable. On the other hand, the influence of the thermophilic microbial complex growing at high temperatures and responsible for the high activities of many enzymes is significant as well. For instance, it is well known that the temperature optimum of endochitinase, an enzyme decomposing randomly the glycoside bonds within the chitin chain, is 40–55°C.

Chitin is a polymer of β-(1,4)-N-acetyl-*D*-glucosamine and a constituent of the external skeleton of fungal cell walls and invertebrates; it is always present in soils. According to the recently published data, high temperatures, radiation, and other adverse factors induce intense synthesis of chitin microfibrils by fungal cells and thickening of the cell wall (a protecting "shield" for fungal cells) [1]. As many authors have repeatedly pointed out, soil microorganisms are active producers of chitinolytic enzymes that are active within a broad temperature range [2, 3]. The question of the formation of the chitinolytic complex of soil microorganisms, as well as its structure and activity, as dependent on the temperature conditions, remains unresolved.

The goal of the present work was to study the taxonomic and functional structure of the chitinolytic microbial complex developing at different incubation temperatures.

MATERIALS AND METHODS

Soil samples collected from the upper humus horizons in various bioclimatic zones, including samples of gley–weakly podzolic and typical gray forest soils, as well as typical chernozem and brown desert–steppe soil of Mongolia, were the subjects of this study. Some of the properties of these soils are listed in Table 1.

To determine the structure of the chitinolytic microbial complex of the studied soils at different temperatures, microbial succession was induced in soil microcosms by moistening and introduction of chitin. A purified chitin preparation (poly-N-acetyl-1,4-β-*D*-glucosamine) was used, manufactured in Germany by ICN Biomedicals, Inc. (MM 770.8, catalogue no. 101334). Since this preparation has been most often mentioned in a number of works by foreign [4, 5] and Russian scientists studying the rates of microbial chitin utilization on various substrates (soil, litter, sewage sediments), it was used in our experiments as well.

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

Table 1. Main properties of the studied soils (*, [6]; **, [7])

Soil	Sampling depth, cm	C_{org}, %	$C_{humic\ acid}/C_{folic\ acid}$	water pH
Gley–weakly podzolic (gley–podzolic*) soil	2–10	0.8	0.5	4.1
Deep typical noncalcareous gray forest soil on mantle loam (gray soil on mantle loam*)	8–20	1.5	0.9	4.9
Typical deeply calcareous heavy loamy chernozem (migrational–segregational deeply calcareous heavy loamy chernozem on loesslike loam*)	5–30	2.9	2.14	6.8
Brown desert–steppe soil** (Mongolia)	2–10	0.64	0.5	8.1

The experiments were carried out at three different temperatures: 5, 27, and 50°C. Due to the fact that the selected temperature range is quite wide and taking into account that the given temperature extremes may be a possible cause of changes in the native complex of soil microorganisms, all soil samples were preincubated at specified temperatures in order to obtain stable microbial communities in the control variants before the beginning of the experiment.

The experiment was performed as follows: weighed portions of soil were placed in penicillin vials, moistened with water up to 60% of the mass of absolutely dry soil, and preincubated for a week at specified temperatures (5, 27, and 50°C). Then, the experimental variants were supplemented with purified chitin (Sigma–Aldrich) (0.6% of the soil mass, i.e., 30 mg/5 g soil), while the controls remained chitin-free. Growth of the microorganisms was then monitored for one month. Dry soil moistened with water was used as a control.

The following parameters weLre used as indicators of chitin degradation by soil microorganisms: the rate of CO_2 emission, dynamics of the cell numbers and bacterial biomass, and length of actinomycete and fungal mycelium, as well as the diversity and abundance of metabolically active prokaryotic cells.

The rate of CO_2 daily emission from the studied soil samples was determined on a Model 3700 gas chromatograph (Russia) equipped with a thermal conductivity detector and a column (3 m) packed with Polysorb-1 with a carrier gas (He) flow of 25 ml/min. CO_2 accumulation in the gas phase of the vials was assayed after 24-h incubation at different temperatures. All soil samples were analyzed in five repeats. The respiration rates were monitored for one month.

Microbial cell numbers, as well as the length of actinomycete and fungal mycelium, were determined by fluorescence microscopy [8]. The number of microbial cells contained in one gram of soil was calculated using the following formula: $N = S_1$ a n/v S_2 c (1), where N is the cell number (mycelium length, μm) in 1 g of soil; S_1 is the preparation area (μm^2); a is the number of cells (mycelium length, μm) in a microscope field (averaging was performed for all preparations); n is the dilution index of the soil suspension (ml); v is the volume of the drop applied to the glass slide (ml); S_2 is the microscope field area (μm^2); and c is the weight of the soil portion (g). The specific weight of microbial cells and the water content in the cell were assumed equal to 1 g/cm^3 and 80%, respectively. The dry biomass index for one bacterial cell (0.1 μm^3) was 2×10^{-14} g; the index for 1 m of actinomycete mycelium (0.5 μm in diameter) was 3.9×10^{-8} g. The dry biomass index for 1 m of fungal mycelium (5 μm in diameter) was 3.9×10^{-6} g [9].

Evaluation of the diversity and the percentage of the metabolically active members of individual phylogenetic groups of chitinolytic microorganisms in the soil samples was carried out by in situ hybridization with 16S rRNA-specific fluorescently labeled oligonucleotide probes (FISH). This technique combines the possibilities for identification and enumeration of individual phylogenetic groups of microorganisms in samples of different nature and has been widely used to study microbial communities in marine and freshwater ecosystems, peats, plant rhizosphere, as well as in many other natural and anthropogenic environments [10–14, 23–25]. In our work, we used a set of probes specific to the representatives of the domains *Archaea* and *Bacteria*, as well as to individual phylogenetic groups of *Bacteria*, which enabled us to analyze the bacterial community of the studied soils. The use of the FISH method has made it possible to enumerate living, metabolically active cells in the soil samples with and without chitin. The preparations were stained with acridine orange in order to determine the total number of microbial cells (including the dormant forms) in the soil samples. The procedure involved the following steps.

(1) The soil suspension (1 : 10) was sonicated at 0.40 A and 22 kHz for 2 min. Microbial cells were separated from large soil particles by thrice-repeated centrifugation at 1000 *g* for 10 min. The suspension obtained after three cycles of processing was centrifuged at 10000 *g* for 10 min. Sterile distilled water was added to the sediment to a volume of 2 ml.

(2) The samples were fixed with paraformaldehyde. The suspension (2 ml) was centrifuged at 10000 *g* for 5 min. The pellet was resuspended in 0.5 ml of phosphate buffer (NaCl, 8.0 g; KCl, 0.2 g; Na_2HPO_4,

Table 2. rRNA-specific oligonucleotide probes used in this study [14]

Probe	Target group	Target site of 16S rRNA	Probe sequence, 5'–3'	Reference
EUB338	*Bacteria*	338–355	CY3 GCT GCC TCC CGT AGG AGT GC(**W**)	[14, 12]
EUB338 I	*Bacteria* (*Planctomycetales*) (*Verrucomicrobiales*)		CY3 GCC (**W**)CC CGT AGG (**W**)GT[a]	
ARCH915	*Archaea*	915–934	CY3 GTG CTC CCC CGC CAA TTC CT	[15]
ALFlb	*Alphaproteobacteria*	19–35	CY3 CGT TCG **Y**TC TGA GCC AG[a]	[16]
ÂÅÒ42a	*Betaproteobacteria*	1027–1043[b]	CY3 GCC TTC CCA CTT CGT TT	[13]
GAM42a	*Gammaproteobacteria*	1027–1043[b]	CY3 GCC TTC CCA CAT CGT TT	[17]
SRB385Db	*Deltaproteobacteria*	385–402	CY3 CGG CGT TGC TGC GTC AGG	[17]
CF319a	*Cytophaga*	319–336	CY3 TGG TCC GTG TCT CAG TAC	[11]
HGC69a	*Actinobacteria*	1901–1918[b]	CY3 TAT AGT TAC CAC CGC CGT[c]	[18]
LGC354A	*Firmicutes*	354–371	CY3 (Y)(G/C)G GAA GAT TCC CTA CTG C[c]	[18]

Note: ([a]) Y = C or T; W = A or T. ([b]) The target molecule is 23S rRNA. ([c]) The probe is used in combination with an unlabeled 5'-TATAGT-TACGGCCGCCGT-3' competitor probe.

1.44 g; NaH_2PO_4, 0.2 g; H_2O, 1.0 l; pH 7.0), supplemented with 1.5 ml of a 4% solution of paraformaldehyde in phosphate buffer, and incubated on a shaker at room temperature for 1.5 h. The fixed material was harvested by centrifugation (10000 *g*, 5 min), washed twice with phosphate buffer, resuspended in ethanol mixed with phosphate buffer (1 : 1), and stored at –20°C until use.

(3) Suspensions of the fixed samples (1 μl) were applied to glass slides with the wells separated by Teflon covering. The fixed specimens were then treated with a lysozyme solution (10 mg in 1 ml of 0.05 M EDTA and 0.1 M *Tris*–HCl; 1 : 1; pH 8.0) to enhance the permeability of bacterial cell walls. The obtained preparations were incubated for 24 h at room temperature and then treated with a series of ethanol solutions (50, 80, and 100%). For hybridization, a set of rRNA-targeted oligonucleotide probes for detection of the representatives of the domains *Bacteria* and *Archaea*, as well as for detection of certain phylogenetic groups within *Bacteria*, was used (Table 2) [14]. The set of Cy3-labeled oligonucleotide probes applied in this study was synthesized by Syntol Co. (Russia). Hybridization of the samples with fluorescently labeled probes was carried out at 46°C according to Amann (1995) [15]. The hybridization conditions used for different probes, as well as formamide concentrations in the hybridization buffer and NaCl concentrations in the washing buffer, are listed in Table 3.

After hybridization, the preparations were additionally stained with aqueous solutions of acridine orange (1 : 10 000; 2–4 min). To remove the excessive fluorochrome, the slides were washed for 10 min in cuvettes with distilled water. The preparations were examined in a ZEISS Mikroskop Axioskop 2 plus fluorescence microscope (Germany) using Filter set 15 to reveal Cy3-labeled oligonucleotide probes and Filter set 09 to reveal cells stained with acridine orange. The numbers of the targeted groups of bacteria in the samples were determined by counting the number of probe-hybridized cells in 50 microscope fields in one well with subsequent calculation of the size of the corresponding populations per 1 g of soil (see Formula (1)).

For pure microbial cultures, emission of carbon dioxide was determined, as was the biomass yield (by spectrophotometry). Chitinase production was determined using chitin-azure (a commercial preparation of dyed chitin) as the substrate [19].

All the soil samples were analyzed in five repeats. The results were statistically examined with the STATISTICA 6.0 software package.

RESULTS AND DISCUSSION

Analysis of the rates of CO_2 production in soil samples supplemented with chitin at various temperatures revealed that the rate of CO_2 emission from chitin-containing soils was higher than from the chitin-free samples at 5, 27, or 50°C, which indicates that a chitinolytic microbial complex developed in the soils within the whole range of temperatures. The highest rate of CO_2 emission (230 μmol C–CO_2/g soil per day; chitin-containing samples) at all the studied temperatures was detected in the chernozem samples, which can be attributed to the huge microbial pool of this soil. The lowest CO_2 emission (50 μmol C–CO_2/g soil per day) was detected in the gley–weakly podzolic and brown desert–steppe soil samples incubated at 5°C.

The study of the dynamics of CO_2 emission from chitin-containing soil samples at different temperatures revealed some patterns: depending on the temperature, the absolute maximum of CO_2 emission was

Table 3. Composition of the hybridization and washing buffers used

	Hybridization buffer					
	Formamide, %	Formamide, μl	H_2O, μl	5 M NaCl, μl	1 M Tris HCl, μl	10% SDS, μl
Eubacteria mix	20	200	600	180	20	1
Archae	30	300	500	180	20	1
Alphaproteobacteria	20	200	600	180	20	1
Betaproteobacteria	35	350	450	180	20	1
Gammaproteobacteria	35	350	450	180	20	1
Deltaproteobacteria	20	200	600	180	20	1
Cytophaga	35	350	450	180	20	1
Actinobacteria	25	250	550	180	20	1
Firmicutes	35	350	450	180	20	1

	Washing buffer (water is added to the volume of 50 ml)			
	5 M NaCl, ml	0.5 M Na-EDTA pH 8, μl	1 M Tris HCl, μl	10% SDS, μl
Eubacteria mix	2.15	500	1	50
Archae	1.02	500	1	50
Alphaproteobacteria	2.15	500	1	50
Betaproteobacteria	0.7	500	1	50
Gammaproteobacteria	0.7	500	1	50
Deltaproteobacteria	2.15	500	1	50
Cytophaga	0.7	500	1	50
Actinobacteria	1.49	500	1	50
Firmicutes	0.7	500	1	50

detected repeatedly at different periods of the experiment. At the lowest temperature (5°C), the peak rate of CO_2 emission was detected by the end of the third week of the experiment; at 27°C, it was detected by the end of the first week; at 50°C, the most intense carbon dioxide emission was observed during the first day of the experiment. By day 30 of the experiment, irrespective of the incubation temperature, the rate of CO_2 emission stabilized, indicating that the chitinolytic succession reached its climax. The control samples were characterized by stable CO_2 emission during the whole period of the experiment due to the fact that they were preincubated and the microbial community reached its climax before the onset of the experiment.

Correct statements concerning chitin decomposition require comparison of the experimental samples with the controls. The absolute values of CO_2 emission from chitin-containing soil samples provide the information on the development dynamics of the chitinolytic microbial succession, but not on the rates of chitin degradation in soils. That is why the coefficient of chitin transformation (CCT) was calculated, which represents the ratio between the rate of CO_2 emission from chitin-containing soils (a) and the rate of CO_2 emission from the control samples (a_0):

$$CCT = a/a_0.$$

The results of CCT calculation indicate that, while at low temperatures, the most intense chitin decomposition occurred in gray forest soil and chernozem, at high temperatures, the rate of chitin decomposition was highest in brown desert–steppe soil (Fig. 1). The moderate temperatures were found to be most favorable for microbial respiration in the gley–weakly podzolic soil samples (Fig. 2). These peculiarities are most likely due to the structure of the natural microbial complex that was initially present in the soil. For instance, in the brown desert–steppe soil samples, the microbial complex was found to be more adapted to high temperatures due to certain climatic factors. On the contrary, in gray forest soil, the microbial complex showed poor growth at high temperatures, but was well adapted to low temperatures.

Similar patterns have previously been observed when studying the ratio between the biomass yields of certain microbial groups in chitin-containing samples and that observed in the control variant. As in the case of CO_2 emission, for more convenient presentation of the data on microbial transformation of chitin in soil at different temperatures, we have calculated the coefficient of microbial response to chitin amendment (CMRC).

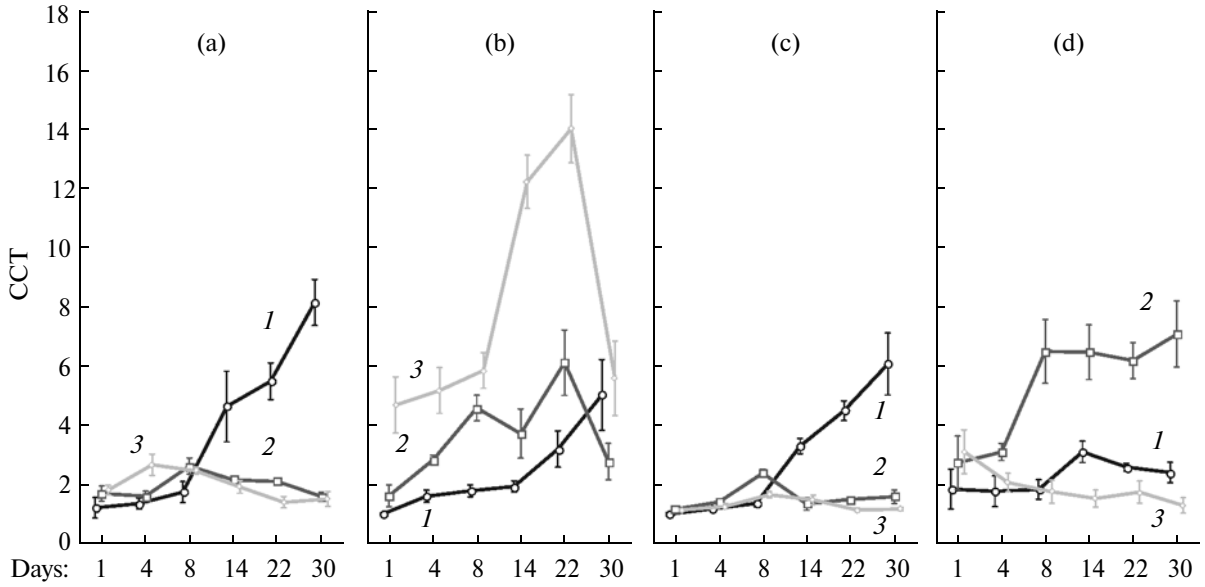

Fig. 1. Dynamics of the coefficient of chitin transformation (CCT) in the studied soils at different temperatures: 5 (*1*), 27 (*2*), and 50°C (*3*); (a), gray forest soil; (b), brown desert-steppe soil; (c), typical chernozem; (d), gley-weakly podzolic soil.

This coefficient represents the ratio between the biomass of certain groups of microorganisms in chitin-containing samples (b) and that in the control variants (b_0):

$$\text{CMRC} = b/b_0$$

The overall coefficient of microbial response to chitin amendment (the ratio between the total microbial biomass in the experimental and control samples) indicates that, at low temperature, the chitinolytic processes were most intense in the gley–weakly podzolic soil samples; at high temperature, these processes were found to be most intense in the brown desert–steppe soil samples (Fig. 3). The lowest CMRC values were observed for gray forest soil and

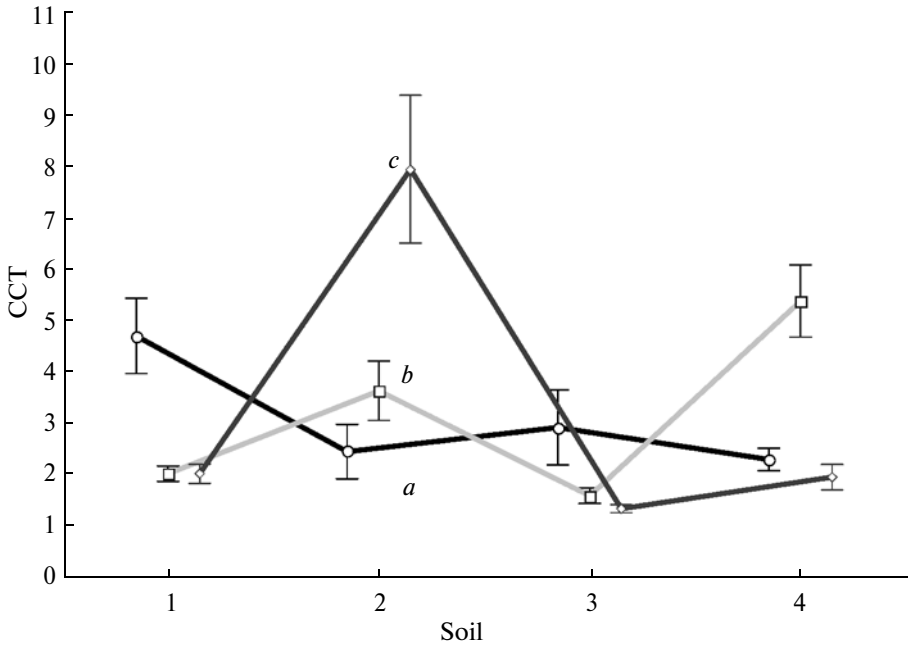

Fig. 2. Average total coefficient of chitin transformation (CCT) in the studied soil samples at different temperatures: 5 (*a*), 27, (*b*), and 50°C (*c*); gray forest soil (*1*), brown desert-steppe soil (*2*), typical chernozem (*3*), gley-weakly podzolic soil (*4*).

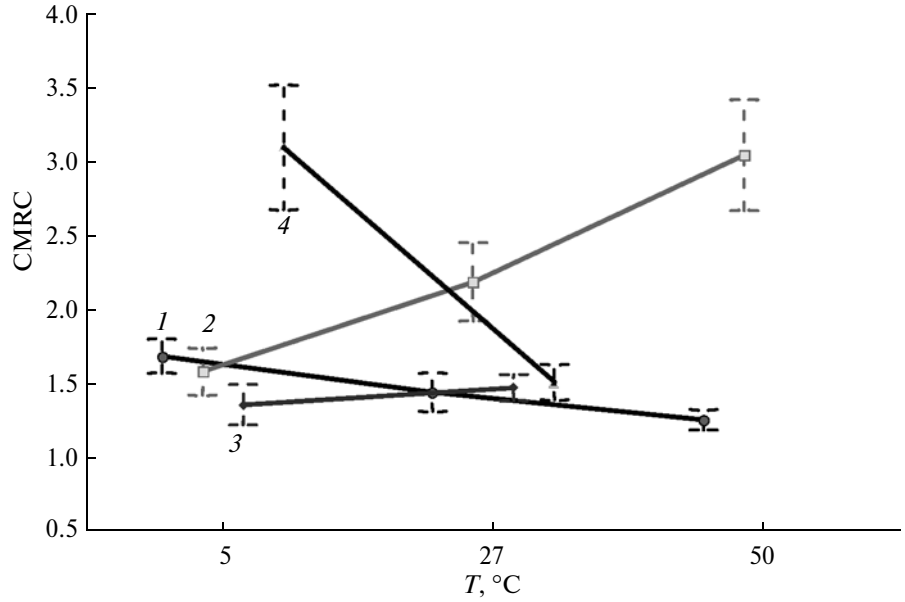

Fig. 3. Average total coefficient of microbial response to chitin amendment (CMRC) in the studied soil samples at different temperatures: gray forest soil (*1*), brown desert-steppe soil (*2*), typical chernozem (*3*), gley-weakly podzolic soil (*4*).

chernozem, which may be due to the concentration of organic matter in these soils affecting the development of microbial succession.

Figure 4 demonstrates the role of specific groups of microorganisms in the degradation of chitin in each soil during incubation at different temperatures. As the temperature decreased, the role of actinomycetes and fungi in chitin transformation in the gray forest and gley–weakly podzolic soils became more important. In the brown desert–steppe soil, mycelial prokaryotes were the most active group of microorganisms at 27 and 50°C. As the temperature increased, the role of unicellular prokaryotes in chitin degradation in this soil became more active as well.

Bacteria played an important role in chitin degradation in the brown desert–steppe soil at high temperatures and in the gley–weakly podzolic soil at low temperatures. At 27°C, unicellular prokaryotes appeared to be equally active in all soil samples. Actinomycetes were found to be the most active chitinolytic microorganisms in all soil samples. Their role in chitin degradation was especially significant in the brown desert–steppe soil at 27 and 50°C, as well as in the gley–weakly podzolic soil at 5°C. Fungi exerted a significant effect on chitin degradation in the gley–weakly podzolic soil at low temperatures. At 27°C, the CMRC values for eukaryotes and unicellular bacteria were virtually the same in all soil samples. At high temperatures, the fungal chitinolytic complex was inactive.

Hence, actinomycetes were found to be the most actively developing microorganisms over the whole temperature range. Bacteria played a significant role in chitin degradation at high temperatures, whereas fungi were most active at low temperatures.

To assess the activity of the chitinolytic community of soil microorganisms, we calculated the specific activity of chitin degradation representing the ratio between the rate of CO_2 emission from the experimental and control samples and the biomass yield in these samples expressed in grams of the CO_2 carbon per gram of the biomass carbon. As a result, it was demonstrated that, of the studied soil samples, the chernozem and gray forest soil samples exhibited the most intense chitinolytic activity at low and moderate temperatures. At high temperatures, the rate of chitin degradation reached its maximum in the brown desert–steppe soil samples. Thus, the confinement of soils to different climatic zones affects the chitinolytic community of soil microorganisms.

Another objective of our study was to analyze the prokaryotic microbial community of the obtained soil samples by in situ hybridization with 16S rRNA-specific fluorescently labeled oligonucleotide probes (FISH). The use of the FISH method has made it possible to enumerate living, metabolically active cells in the soil samples with and without chitin.

On the whole, the total number of cells hybridized with the universal *Bacteria*- and *Archaea*-specific probes varied from 20 (chernozem) to 90% (gley–weakly podzolic and brown desert–steppe soils) of the total number of cells revealed by staining with acridine orange. The relatively low ratio of the cells in the chernozem sample identified using the universal probes may be explained by the following hypotheses. The first one suggests that a considerable proportion of prokaryotic organisms in the chernozem samples are

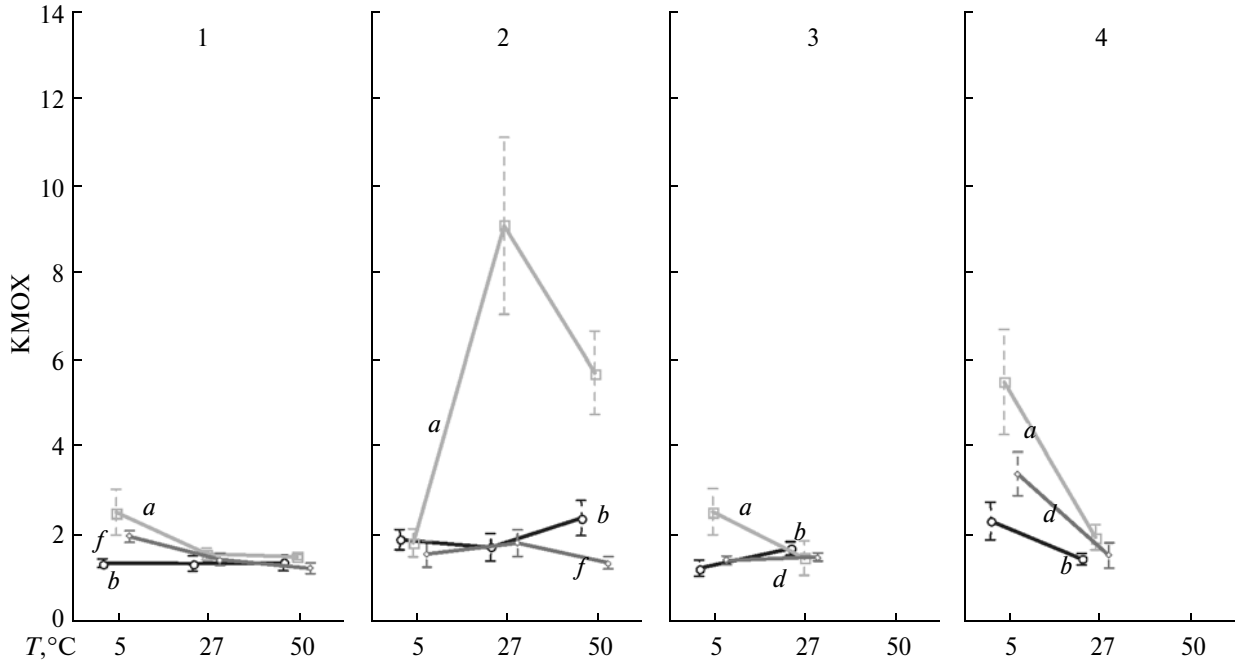

Fig. 4. Coefficient of microbial response to chitin amendment (CMRC) calculated for different groups of microorganisms (*b*, bacteria; *a*, actinomycetes; *f*, fungi) from the studied soil samples at different temperatures: gray forest soil (1), brown desert-steppe soil (2), typical chernozem (3), gley-weakly podzolic soil (4).

dormant or metabolically inactive cells. The second hypothesis suggests that the representatives of some bacterial and archaeal phylogenetic groups that cannot be identified using EUB338-mix probes are present in these soil samples. The majority of cells identified in chernozem were represented by small cells (0.5 μm in length). Such cells comprised up to 50–65% of the total number of the cells detected in chernozem samples by staining with acridine orange. We failed to detect these microorganisms with oligonucleotide probes, possibly due to the fact that the targeted sequences of these organisms did not coincide with the probes or due to the low intensity of the fluorescent signal of the cells hybridizing with these probes. In addition, the granulometric composition of the soil samples affects the results of cell identification. High concentration of the silt fraction in chernozem ensures a higher level of cell adhesion on silt particles as compared to soils with a lighter granulometric composition and, consequently, a decrease in the amount of identified cells. In the chitin-containing soil samples (as compared to the control samples), the number of metabolically active representatives of the domains *Bacteria* and *Archaea* was high at all temperatures (Fig. 5). The largest numbers of archaea were observed in the chitin-containing samples of brown desert–steppe soil at high temperatures.

For identification and quantitative assessment of the phylogenetic groups of eubacteria inhabiting the above-mentioned soils, we used a set of seven group-specific oligonucleotide probes that are most commonly used in modern molecular ecological studies. The presumable detection spectrum of these probes includes representatives of several phylogenetic lineages of the domain Bacteria, namely, the phyla *Proteobacteria, Bacteroidetes, Actinobacteria*, and *Firmicutes*. The probes applied in the present work targeted four of the five recognized classes of the phylum *Proteobacteria: Alpha-, Beta-, Gamma-*, and *Deltaproteobacteria*.

Enumeration of individual phylogenetic groups of the domain *Bacteria* in the chitin-containing samples of gley–weakly podzolic and brown desert–steppe soils at 27°C revealed an increase in the number of bacteria belonging to the phylogenetic lineages *Actinobacteria, Firmicutes*, and *Bacteroidetes*, as well as in the number of members of the phylum *Gammaproteobacteria* (Fig.6). Moreover, in the chitin-containing samples of brown desert–steppe soil, an increase in the number of *Betaproteobacteria* was detected.

These findings can be attributed to the fact that some representatives of these groups exhibit high hydrolytic activity and are able to utilize difficult-to-degrade polymers.

In the chitin-containing samples of gley–weakly podzolic and brown desert–steppe soils (27°C), the eubacteria identified using the EUB338-mix probe comprised 78 and 59% of the total bacterial counts, respectively (Fig. 7).

In the chitinolytic microbial complex of gley–weakly podzolic soil, equal proportions of *Actinobacteria, Firmicutes*, and *Proteobacteria* were present

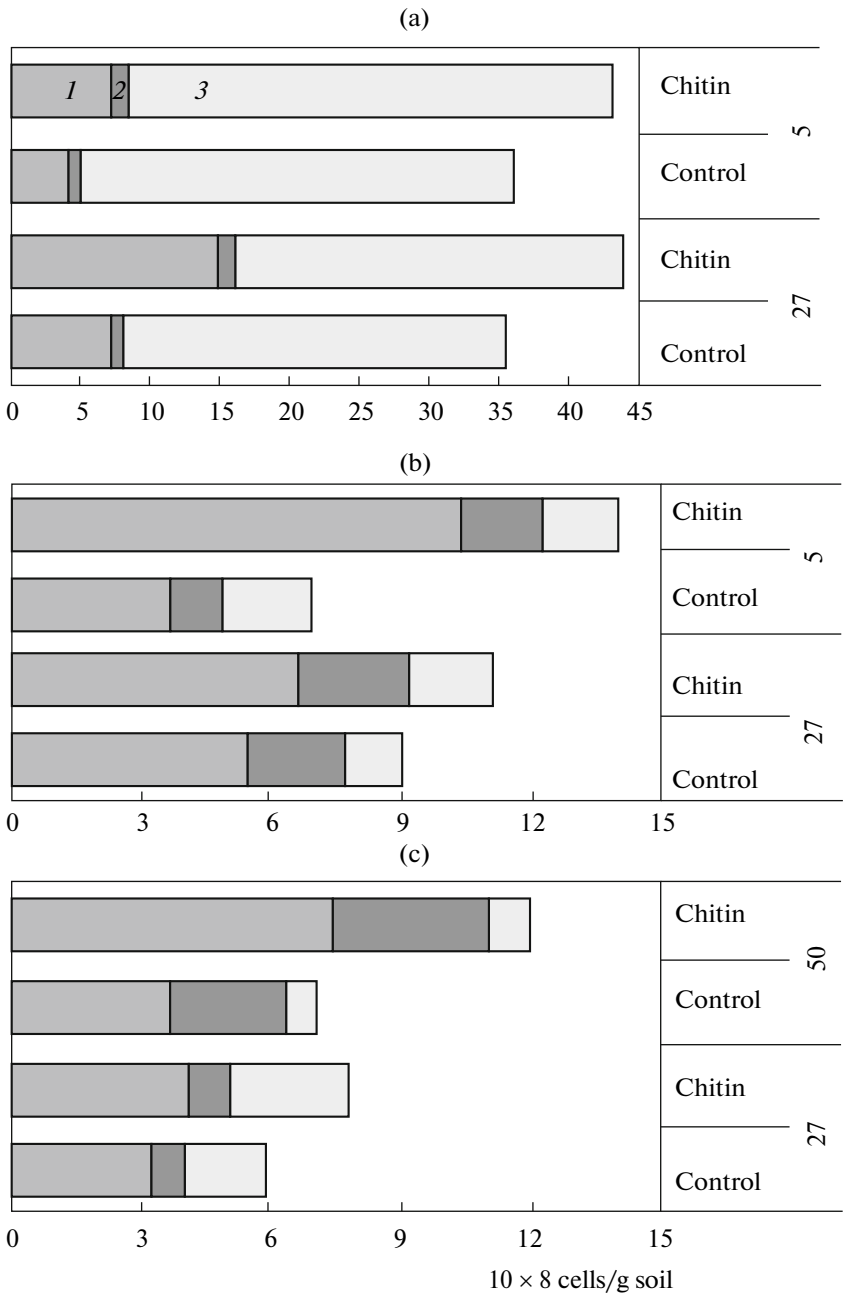

Fig. 5. The percent ratio between eubacteria (*1*), archaea (*2*), and unidentified prokaryotic microorganisms (*3*) in the studied soil samples with and without chitin at different temperatures: typical chernozem (a); gley-weakly podzolic soil (b) and brown desert-steppe soil (c).

among the cells revealed by hybridization with group-specific oligonucleotide probes. In the brown desert–steppe soil samples, an increase in the number of *Proteobacteria* was detected, whereas, at the same time, the numbers of *Firmicutes* and *Actinobacteria* decreased.

At low temperatures (5°C), the number of microorganisms belonging to the phyla *Proteobacteria* and *Firmicutes* increased in the chitinolytic microbial complex of the samples of chernozem and gley–weakly podzolic soil, whereas the number of gram-positive bacteria with the high DNA G + C base content belonging to the phylum *Actinobacteria* decreased as compared to their number detected in these samples at 27°C.

In the chitinolytic microbial complex of brown desert–steppe soil, an increase in the number of unidentified forms of eubacteria was detected at 50°C. It should be noted that the number of gram-positive bacteria with a high DNA G + C base content increased

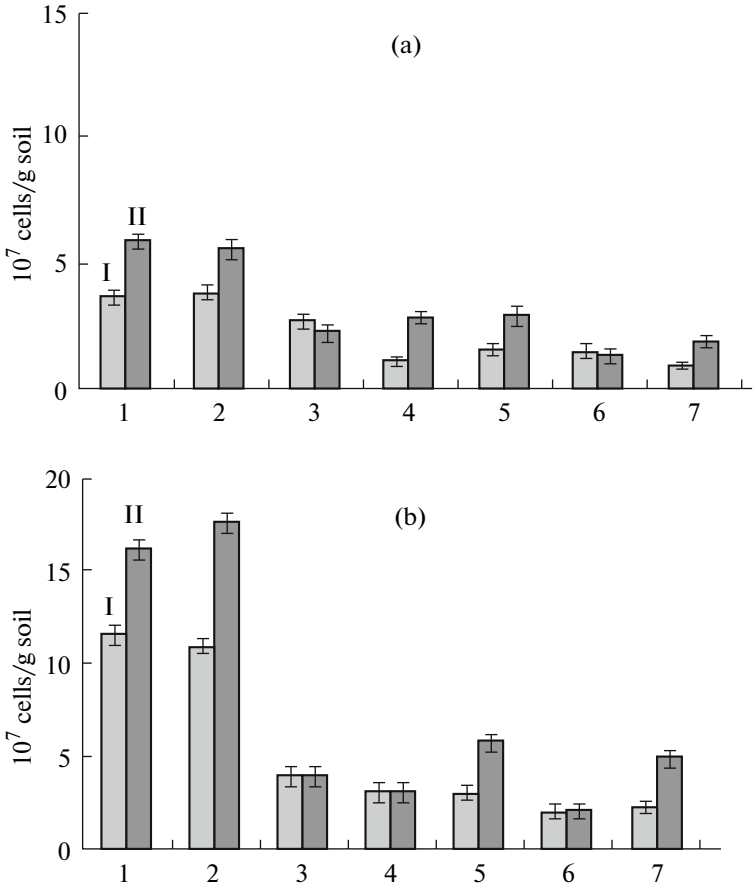

Fig. 6. The numbers of individual phyla of the domain *Bacteria* in the microbial complex of the chitin-containing (II) and chitin-free (I) brown desert-steppe (a) and gley-weakly podzolic (b) soil samples at 27°C: (1) *Actinobacteria*, (2) *Firmicutes*, (3) *Alphaproteobacteria*, (4) *Betaproteobacteria*, (5) *Gammaproteobacteria*, (6) *Deltaproteobacteria*, and (7) *Barteroidetes*.

in all chitin-containing samples. More attention should be paid to the growth of mycelial forms of actinobacteria, the biomass yield of which increased by one order of magnitude in the chitin-containing soil samples.

Thus, by using the FISH method, the structural differences in the chitinolytic microbial complexes of the studied soils, manifesting themselves at different temperatures, were determined.

Among the potential agents of microbial degradation of chitin in soil at all the studied temperatures revealed by FISH, members of the phyla *Actinobacteria* and *Firmicutes* prevailed. These groups of microorganisms have been most often mentioned as the main agents of organic matter decomposition in aquatic and terrestrial ecosystems [20, 21]. At low temperatures, the number of *Actinobacteria* in the chitinolytic complex of soil microorganisms decreased, whereas a significant increase in the number of mycelial forms of *Actinobacteria* occurred at high temperatures. The abundance of the growing physiologically active cells of *Bacteroidetes, Gammaproteobacteria*, and *Betaproteobacteria* in chitin-containing soil samples indicates that these microorganisms actively participate in the degradation of this polymer within a broad temperature range.

In our previous works [19, 22] dealing with the taxonomic position of the most active lytic microorganisms, we demonstrated that mycelial actinobacteria and representatives of *Gammaproteobacteria* prevailed in the chitinolytic complexes on solid nutrient media, which was confirmed by the results of fluorescence in situ hybridization. The cultural, morphological, and physiological properties of the detected actinomycetes, as well as their 16S rRNA sequences, indicate that these microorganisms belong to the genus *Streptomyces*. At moderate, high, and low temperatures, they were found to be most closely related to *Str. wedmorensis, Str. roseolilacinus*, and *Str. mirabilis*, respectively. According to phylogenetic analysis of the 16S rRNA gene sequences, the predominant bacterial strain in the chitinolytic microbial complex of brown desert–steppe soil at 50°C was *Silanimonas lenta*, belonging to the recently discovered genus of the family *Xanthomonadaceae* of *Gammaproteobacteria*. These results correspond to the data obtained by means of

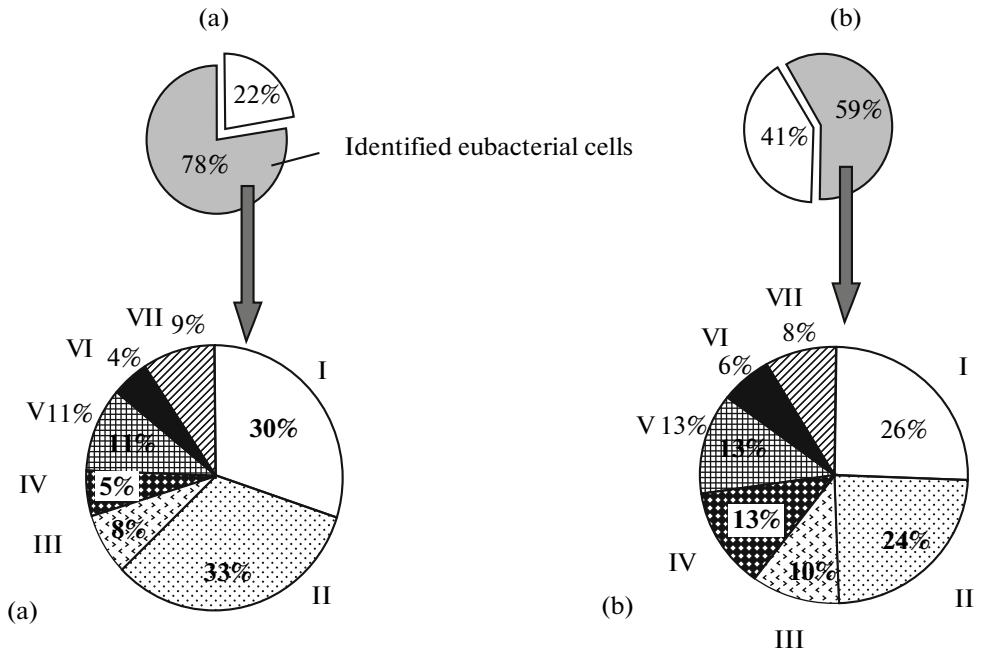

Fig. 7. The percent ratio between the representatives of different phylogenetic groups revealed using a set of group-specific oligonucleotide probes and that of the total bacterial numbers of the identified eubacteria in the chitinolytic complexes of the gley-weakly podzolic (a) and brown desert-steppe (b) soils at 27°C: (I) *Actinobacteria*, (II) *Firmicutes*, (III) *Alphaproteobacteria*, (IV) *Betaproteobacteria*, (V) *Gammaproteobacteria*, (VI) *Deltaproteobacteria*, and (VII) *Bacteroidetes*.

FISH on the increasing number of *Gammaproteobacteria* in chitin-containing soil.

Hence, as a result of this work, it was demonstrated that the most intense chitinolytic activity was detected in chernozem and gray forest soil at low and moderate temperatures. At high temperatures, the rates of chitin degradation were highest in the brown desert–steppe soil samples, due to the structure of the chitinolytic microbial complex. It was demonstrated for the first time that, in soils incubated at high temperatures, prokaryotes are the main chitin degraders, whereas fungi more actively participate in chitin decomposition at low temperatures. The use of the FISH method for chitin-containing soil samples (as compared to the control variants) revealed an increase in the number of metabolically active representatives of the domains *Bacteria* and *Archaea* at all the experimental temperatures. The highest number of archaea was observed in chitin-containing samples of brown desert–steppe soil at high temperatures. At all the experimental temperatures, eubacteria of the phyla *Actinobacteria, Firmicutes*, and *Bacteroidetes*, as well as of the classes *Beta-* and *Gammaproteobacteria*, are involved in the process of chitin degradation. A significant increase in the number of the mycelial forms of *Actinobacteria* occurs at high temperatures. A relationship was established between the taxonomic position of the dominant members of the chitinolytic complex of soil microorganisms isolated in pure cultures and the dominant phylogenetic groups and sequence types.

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