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SOME FEATURES OF ENZYME ACTIVITY IN DIFFERENT STRAINS OF THE *BACILLUS* GENUS ISOLATED FROM PERMAFROST

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The kinetics of growth and activity of enzymes in bacterial strains of the *Bacillus* genus isolated from permafrost are studied as a function of incubation temperatures. Viable bacteria were found in permafrost in the area of Tarko-Sale in northern West Siberia. The selected permafrost core samples are Upper and Middle Pleistocene alluvial and lacustrine deposits of marine terrace IV ($mIII_1$, mII_{2-4}). The *Bacillus* spp. strains change notably in growth kinetics and enzyme activity as temperatures vary from 5 to 45 °C, which is evidence of their adaptation ability. Activity of catalase, dehydrogenase, amylase, protease and lipase enzymes is high at low temperatures in most of the analyzed bacterial strains, which has important biotechnological implications.

Permafrost, Bacillus bacteria, extremozymes, growth kinetics, enzyme activity

INTRODUCTION

Microorganisms are ubiquitous in ice or any frozen rock. When exposed to extreme conditions, e.g., low temperatures, microbiota develops exceptional abilities, specifically, activity of certain enzymes. Thus, studying microbial life in permafrost is of particular theoretical and practical interest.

Recently a wealth of evidence has been gained on adaptation of microorganisms to extreme environments, especially, on biochemical and physiological changes in bacterial cells and their metabolism in response to cold [*Nikrad et al., 2016; Baraúna et al., 2017*]. Metabolic activity was reported for bacteria in permafrost exposed to –20 °C which reach the stationary phase in less than a year after freezing [*Rivkina et al., 2000*]. Microorganisms that thrive in cold environments become the source of new metabolites and proteins, as well as producers of enzymes stable within a large range of physicochemical conditions (extremozymes) [*MacElroy, 1974; Coker, 2016*]. Investigation into the ability of microorganisms to live under low temperatures opens new avenues in their industrial, agricultural, medical, and biotechnological uses. In this respect, Arctic microbiota is a renewable cryo-biological resource of special value [*Melnikov and Gennadinik, 2011; Stan-Lotter and Fendrihan, 2011; Melnikov, 2012; Feller, 2013*]. Most of relevant publications address biodiversity of microbial communities in various objects of the cryosphere [*Cameron, 1972; Gilichinskiy et al., 1989; Zvyagintsev et al., 1990; Vorobyova et al., 1997; Hansen et al., 2007*], but their practical potential, including the enzyme activity, has received less attention.

Enzymes in psychrophilic and psychrotolerant microorganisms are of practical interest because they remain active within larger temperature and pH ranges than those currently employed in biotechnology. Thus, they can reduce risks of unwanted chemical reactions that may occur at warmer temperatures [*Bowman et al., 2005; Gesheva and Vasileva-Tonkova, 2012; Vester et al., 2014*].

High catalytical activity at low temperatures is known for amylase, lipase, protease, lactamase, cellulase, xylanase, chitinase, and pectinase enzymes. Cold-active proteases have been a common component in food, pharmaceutics, and detergents [*Kasana, 2010; Joshi and Satyanarayana, 2013*]. Food, textile, and paper industries use amylases, the second significant group of enzymes [*Konsoula and Liakopoulou-Kyriakides, 2007; Kuddus et al., 2012*], as well as lipases useful for their bio-catalytical properties [*Petrovskaya et al., 2012; Maiangwa et al., 2015*].

Comparison of enzyme activity in meso- and psychrophilic bacteria [*D'Amico et al., 2002*] has shown that the former have low or sometimes zero activity at low temperatures. Currently only 1–2 % of microorganisms have found commercial applications, among which only few extremophiles [*Gome and Steiner, 2004*].

Revolutionary advance in molecular biology leads to large-scale production of many valuable extremozymes involved in bio-catalytical processes. However, in spite of great recent progress, the available knowledge of physiology, metabolism, enzyme activity, and genetics of extremophilic microbiota remains quite limited.

This study focuses on the effect of temperature on growth kinetics and enzyme activity in some strains of the *Bacillus* genus isolated from permafrost.

MATERIALS AND METHODS

We investigated *Bacillus* bacteria isolated previously from permafrost in the Tarko Sale area of northern West Siberia [*Domanskaya et al., 2015*]. Nine pure cultures of *Bacillus* spp. were identified by microbiological analysis. The identification of species was based on cultural-morphological, physiological, and biochemical criteria and was checked by analysis of 16SpRNA gene fragment sequences in PCR reactions with the primers 8f (AGA GTT TGA TCC TGG CTC AG), 926r (CCG TCA ATT CCT TTR AGT TT) and 1492r (GGT TAC CCT TGT TAC GAC TT). The sequencing was performed at the State Research Institute of Genetics (Moscow). The obtained nucleotide sequences were compared with those from the international databank NCBI using the Basic Local Alignment Search Tool (BLAST) [http://blast.ncbi.nlm.nih.gov/Blast.cgi]. We also applied *ClustalW2* for sequence alignment of the 16SpRNA genes and *MEGA 5.2* for phylogenetic analysis.

The results were checked against data for the strain *Bacillus* sp. 3M (VKPM B-10130) isolated from permafrost in Yakutia, courtesy of researchers from the Tyumen Science Center [*Brouchkov et al., 2009*].

The *Bacillus* sp. bacterial strains were cultivated in shaker incubators, at 180 rpm, in 100 mL Erlenmeyer flasks with 50 mL of nutrient agar medium consisting of 1.0 g/L K_2HPO_4; 0.5 g/L $MgSO_4 \cdot 7H_2O$; 2.0 g/L $(NH_4)_2SO_4$; 2.0 g/L peptone; 5.0 g/L yeast extract; and 5.0 g/L glycerin. An overnight culture grown in a medium of the same composition was used for inoculation. Incubation at 22 and 45 °C temperatures was in a thermostated *BioSan ES-20* shaker incubator (Latvia), with sampling every hour. At 5 °C, the bacteria were cultivated in a *Polair* cooling chamber (Russia), with sampling every three hours. The concentration of cells in a suspension was estimated according to optical density in a *UNICO-2800* spectrophotometer at 540 nm, with 10 mm thick cuvettes. The kinetics of periodic bacterial growth was studied following the recommendations of *Pirt* [*1975*].

All *Bacillus* strains were investigated for potential catalase, dehydrognease, protease, amylase, and lipase activity by turbidity measurements of liquid cultures. Catalase activity was detected by colorimetric assay [*Koroluk et al., 1988*] based on the ability of H_2O_2 to produce a dye complex with ammonium molybdate having the maximum absorbance at 405 nm. Dehydrogenase activity was determined by a modified technique of *Romanenko and Kuznetsov* [*1974*] proceeding from the ability of dehydrogenase to reduce 2,3,5-triphenyl tetrazolium chloride (TTC) at the account of dehydration to purple 2,3,5-triphenylformazan (TFF). The formazan content was evaluated according to optical density by 485 nm spectrophotometry. The activity of proteases was measured by the method of *Pant et al.* [*2015*] from the amount of amino acids that form by protein-dye binding. Amylase activity was estimated by the method of Smith and Roy in a modification of Manning and Campbell [*Netrusov et al., 2005*], by colorimetry (rate of dye change with I), on a spectrophometer at 620 nm. Lipase activity was determined by titrimetric assay using an olive oil substrate solution [*Gracheva et al., 1982*]. The method implies alkaline titration of free fatty acids released from olive oil. Protein in liquid cultures was determined by the protein dye-binding method of *Bradford* [*1976*]. All measurements were run in triplicate. The experiment results were processed using the R 3.2.0 software for data analysis and visualization (Free Software Foundation, Inc Boston, MA).

RESULTS AND DISCUSSION

Bacterial strains isolated from permafrost were identified as the *B. megaterium* (3 specimens), *B. cereus* (4 specimens), *B. simplex* (1 specimen) and *B. subtilis* (1 specimen) species. Phylogenetic analysis of the strains 1562 TS, 875 TS, 1257 TS and 630 TS showed their closest affinity with *B. cereus* (99 % level of sequence similarity). The strains 206 TS, 312 TS and 629 TS are genetically similar to *B. megaterium* (97, 99 and 98 % similarity, respectively). *B. simplex* and *B. subtilis* are the closest neighbors of 948P-1 and 948P, respectively, with a similarity of 99 and 98 % (Table 1). The effect of incubation temperatures on growth kinetics of the *Bacillus* strains was estimated at periodic conditions using lag-phase inoculation, thus missing the spore growth phase. The periodic bacterial growth is known to consist of four main phases: lag phase (adaptation to growth conditions), log or exponential phase (growth rate acceleration), stationary phase (growth rate deceleration under limiting factors) and death phase (population decline). Bacterial synthesis of secondary metabolites increases in the end of the log phase and reaches its maximum in the stationary phase [*Timmusk et al., 1999*].

The analyzed strains responded in different ways to temperature variations. Growth began right after inoculation, and the lag phase lasted from 30 min to 4 hr depending on incubation temperature. At 45 °C it was 30 min long in most of cultures. Then the cells grew rapidly for 3.5 hr and reached the stationary

phase in 4 hr (Fig. 1, *a*). At 22 °C, the lag phase became 2–4 hr longer; it began in 4–6 hr and lasted 3.7 hr on average. The cultures reached the stationary phase in 10–12 hr (Fig. 1, *b*) and their biomass measured by optical density became almost 4 times greater by that time than at 45 °C.

The growth rates at 5 °C were slower in all phases: the lag phase lasted from 15 to 68 hr, i.e., the adaptation was much longer than at warmer incubation temperatures; the log phase lasted from 27 to 117 hr on average, and the stationary phase began after 72 hr of incubation. The maximum cell density was observed in *B. simplex* 948P-1 and *B. meganterium* 312 (Fig. 1, *c*).

The obtained data for the analyzed strains were used to calculate the parameters of cell growth kinetics: the growth rate μ and the generation time *g* (Table 2). The cultures differ in growth rates: μ is the highest in the strain *B. simplex* 948P-1 at an incubation temperature of 22 °C and the lowest in *B. megaterium* 312 and *B. subtilius* 948P at 5 °C, the average being $\mu = 0.048\ hr^{-1}$, $g = 12.1$ hr. For instance, the generation time of the Antarctic strain *Pseudoalteromonas haloplanktis* is 4 hr at an incubation temperature of 4 °C [*Piette et al., 2011*] while the respective time for *Psychromonas ingrahamii* isolated from the Arctic marine ice is 12 hr at 5 °C [*Rivkina et al., 2000*]. Microorganisms incubated at 45 °C exhibited high growth rates but long generation times and quite low biomass accumulation.

Thus, the growth of the *Bacillus* strains depends on incubation temperature. The revealed growth patterns allowed choosing the optimal temperatures that maintain the highest production. The strains reached the maximum cell density at both 5 and 22 °C, but in a longer time for 5 °C. No significant biomass increase was observed during incubation at 45 °C.

Table 1. **Identification of cultivable bacteria isolated from permafrost in West Siberia (Dremuchee deposit)**

No.	Depth, m	Strain	Taxonomy	Similarity 16SpRNA, %
1	2.5	1562 TS	*Bacillus cereus*	99
	2.5	875 TS	*Bacillus cereus*	99
	10.0	206 TS	*Bacillus megaterium*	97
	30.3	1257 TS	*Bacillus cereus*	99
2	4.2	312 TS	*Bacillus megaterium*	99
	10.0	629 TS	*Bacillus megaterium*	98
	10.0	630 TS	*Bacillus cereus*	99
	12.3	948-P1 TS	*Bacillus simplex*	99
	12.3	948P TS	*Bacillus subtilis*	98

The strain *B. cereus* was previously considered to be a mesophile, but psychrotrophic forms which can grow at temperatures between 7 and 30 °C were also reported [*Francis et al., 1998; Montanhini et al., 2013*]. Our results showed psychrotrophic origin of the strains *B. cereus* 875, *B. cereus* 1257, and *B. cereus* 630.

Bacterial synthesis of protein depends on environmental limiting factors, especially, temperature which controls biomass accumulation and rates of metabolic reactions.

The strains *B. megaterium* 206 and *B. cereus* 1562 showed the highest protein content in the liquid cultures at 22 °C: 21.45 μg/mL and 21.42 μg/mL, re-

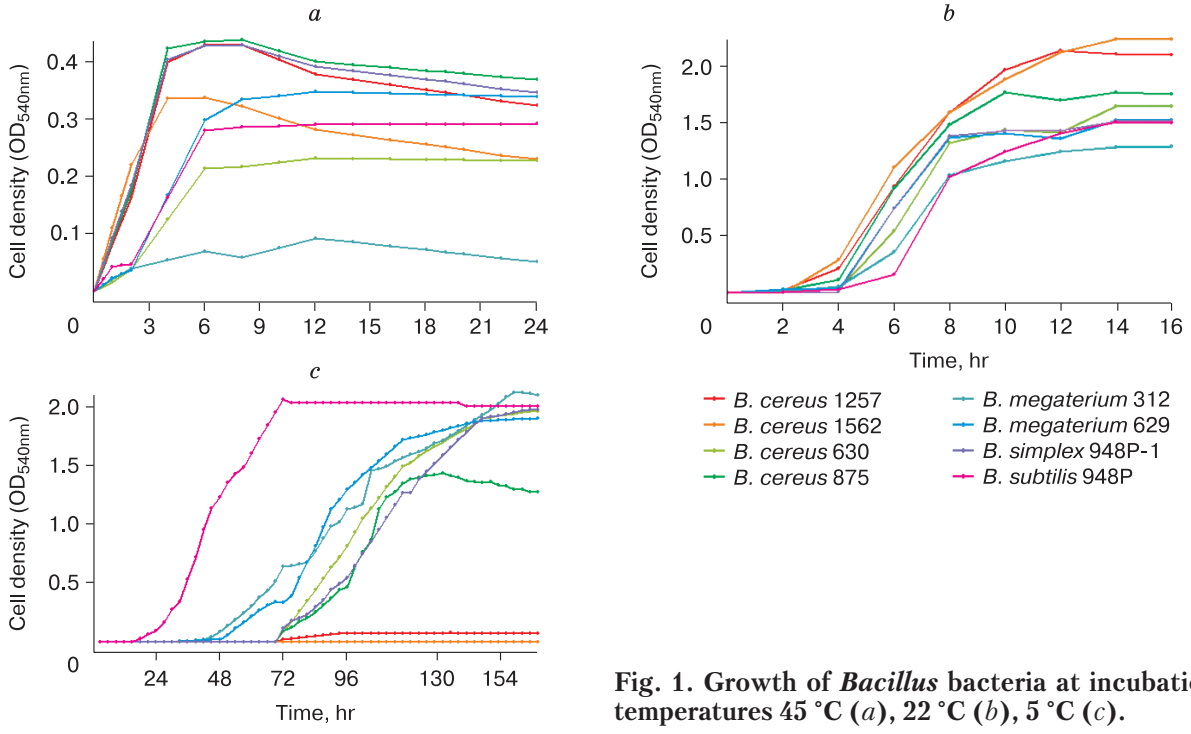

Fig. 1. Growth of *Bacillus* bacteria at incubation temperatures 45 °C (*a*), 22 °C (*b*), 5 °C (*c*).

Table 2. **Kinetic growth parameters of *Bacillus* strains at different incubation temperatures (*T*)**

Strain	*T* = 5 °C			*T* = 22 °C			*T* = 45 °C		
	μ, hr^{-1}	*g*, hr	CD_{max}	μ, hr^{-1}	*g*, hr	CD_{max}	μ, hr^{-1}	*g*, hr	CD_{max}
B. cereus 875	0.064	10.828	1.427	0.641	1.081	1.765	0.676	1.025	0.438
B. cereus 1257	0.055	12.600	0.072	0.372	1.862	2.132	0.657	1.054	0.430
B. cereus 1562	–	–	0.000	0.505	1.372	2.231	0.517	1.340	0.338
B. megaterium 206	0.040	17.325	1.962	0.682	1.019	1.269	0.906	0.764	0.293
B. megaterium 312	0.034	20.382	2.102	0.529	1.310	1.284	0.185	3.745	0.092
B. cereus 630	0.063	11.002	1.945	0.946	0.756	1.640	0.433	1.600	0.232
B. subtilis 948P	0.034	20.382	1.459	0.915	0.757	1.502	0.451	1.536	0.292
B. megaterium 629	0.063	11.001	1.945	1.000	0.693	1.520	0.529	1.310	0.348
B. simplex 948P-1	0.082	8.6620	2.02	1.362	0.509	1.508	0.453	1.529	0.430

Note. μ = specific growth rate of cell culture, hr^{-1}; *g* = cell generation time, hr; CD_{max} = maximum bacterial cell density in suspension ($OD_{540\ nm}$).

spectively. Protein synthesis reduced during incubation at 45 °C, and its average content in the liquid was as low as 4.06 μg/mL. The maximum protein contents were observed in the *B. simplex* 948P-1 (25.80 μg/mL), *B. subtilis* 948P (16.80 μg/mL), and *Bacillus* sp. 3M (13.35 μg/mL) strains incubated at 5 °C. Note that the amount of protein in the liquid cultures correlated with accumulation of biomass.

The temperature effect on the enzyme activity of the *Bacillus* strains is summarized in Table 3.

Table 3. **Enzyme activity of studied *Bacillus* strains**

Strain	Temperature, °C	Catalase (U mg protein)	Dehydrogenase (mg/(mL·day))	Lipase (μmol/(mL·min))	α-amylase (U/(mL·min))	Protease (U/(mL·30 min))	Protein (μg/mL)
B. cereus 630	5	21.49 ± 2.36	0.21 ± 0.01	3.04 ± 0.28	0.04 ± 0.00	0.03 ± 0.00	108.36 ± 16.36
	22	9.73 ± 0.99	0.13 ± 0.01	0.74 ± 0.07	0.28 ± 0.02	0.12 ± 0.01	118.29 ± 17.86
	45	9.13 ± 0.82	0.01 ± 0.00	0.41 ± 0.04	0.01 ± 0.00	0.01 ± 0.00	78.64 ± 12.03
B. cereus 875	5	11.64 ± 1.20	0.15 ± 0.01	2.66 ± 0.28	0.39 ± 0.02	0.17 ± 0.02	223.06 ± 35.02
	22	4.57 ± 0.50	0.09 ± 0.01	1.22 ± 0.12	0.24 ± 0.01	0.20 ± 0.02	92.01 ± 14.63
	45	12.35 ± 1.36	0.01 ± 0.00	0.36 ± 0.04	0.02 ± 0.00	0.05 ± 0.01	94.27 ± 14.42
B. cereus 1257	5	23.07 ± 2.08	0.11 ± 0.01	1.53 ± 0.16	0.24 ± 0.01	0.08 ± 0.01	94.73 ± 14.87
	22	0.62 ± 0.06	0.18 ± 0.01	0.94 ± 0.10	0.09 ± 0.00	0.10 ± 0.01	62.30 ± 9.84
	45	3.86 ± 0.31	0.01 ± 0.00	0.54 ± 0.05	0.03 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
B. cereus 1562	5	10.46 ± 1.15	0.15 ± 0.01	0.83 ± 0.08	0.08 ± 0.00	0.06 ± 0.01	129.27 ± 19.64
	22	3.82 ± 0.39	0.09 ± 0.01	1.21 ± 0.11	0.11 ± 0.01	0.09 ± 0.01	118.21 ± 18.80
	45	6.43 ± 0.45	0.01 ± 0.00	0.85 ± 0.08	0.06 ± 0.00	0.17 ± 0.02	85.48 ± 13.85
B. megaterium 312	5	20.58 ± 2.02	0.26 ± 0.02	1.46 ± 0.13	0.08 ± 0.00	0.01 ± 0.00	97.76 ± 14.18
	22	10.62 ± 0.85	0.30 ± 0.02	1.33 ± 0.12	0.19 ± 0.01	0.18 ± 0.02	76.55 ± 11.41
	45	12.24 ± 1.35	0.00 ± 0.00	0.45 ± 0.04	0.23 ± 0.01	0.07 ± 0.01	88.67 ± 12.59
B. megaterium 629	5	14.49 ± 0.72	0.08 ± 0.01	1.40 ± 0.12	0.32 ± 0.02	0.00 ± 0.00	67.45 ± 10.86
	22	4.34 ± 0.39	0.16 ± 0.01	0.70 ± 0.06	0.11 ± 0.00	0.01 ± 0.00	114.12 ± 18.15
	45	2.14 ± 0.22	0.00 ± 0.00	0.95 ± 0.09	0.03 ± 0.00	0.10 ± 0.01	73.82 ± 11.22
B. megaterium 206	5	26.79 ± 2.95	0.18 ± 0.01	2.21 ± 0.22	0.17 ± 0.01	0.14 ± 0.02	102.30 ± 15.14
	22	3.06 ± 0.28	0.08 ± 0.01	2.00 ± 0.22	0.05 ± 0.00	0.34 ± 0.04	67.76 ± 10.91
	45	7.65 ± 0.73	0.00 ± 0.00	0.13 ± 0.01	0.03 ± 0.00	0.10 ± 0.01	96.85 ± 15.79
B. subtilis 948P	5	17.44 ± 1.92	0.15 ± 0.01	2.16 ± 0.22	0.06 ± 0.00	0.09 ± 0.01	125.03 ± 19.75
	22	5.87 ± 0.60	0.05 ± 0.00	1.80 ± 0.16	0.05 ± 0.00	0.22 ± 0.02	127.15 ± 20.09
	45	5.63 ± 0.61	0.01 ± 0.00	1.75 ± 0.18	0.04 ± 0.00	0.01 ± 0.00	84.12 ± 13.21
B. simplex 948P-1	5	21.44 ± 2.36	0.33 ± 0.03	2.63 ± 0.28	0.12 ± 0.01	0.20 ± 0.02	114.27 ± 17.21
	22	7.82 ± 0.66	0.03 ± 0.00	0.36 ± 0.04	0.13 ± 0.01	0.09 ± 0.01	150.18 ± 24.18
	45	0.73 ± 0.08	0.00 ± 0.00	0.55 ± 0.05	0.06 ± 0.00	0.08 ± 0.01	60.94 ± 8.90
Bacillus sp. B-10130	5	18.13 ± 1.60	0.06 ± 0.00	1.72 ± 0.14	0.18 ± 0.01	0.09 ± 0.01	168.67 ± 24.96
	22	14.16 ± 1.50	0.03 ± 0.00	1.53 ± 0.12	0.14 ± 0.01	0.25 ± 0.03	98.42 ± 14.66
	45	2.94 ± 0.29	0.00 ± 0.00	0.56 ± 0.05	0.12 ± 0.01	0.11 ± 0.01	51.24 ± 7.22

Most of microorganisms respond to extreme conditions by increasing the activity of antioxidant protection enzymes. High catalase activity allows bacteria to survive at low positive and negative temperatures. Catalase is a psychrophilic enzyme active between 0 and 30 °C [*Gerday et al., 1997*].

Catalase activity in the studied strains varied from 0.73 to 12.35 U/mg of protein at 45 °C and from 0.62 to 14.16 U/mg at 22 °C, but was times higher in cultures grown at 5 °C (10.46 to 26.79 U/mg on average). Increasing catalase activity at low positive temperatures is a common feature in psychrophilic microorganisms [*Frank et al., 1963; Yumoto et al., 2000*; *Kimoto et al., 2012*].

The growth of psychrophilic and psychrotolerant bacteria at low positive and negative temperatures is associated with increasing energy demands which induces synthesis of dehydrogenase involved into dehydration and related synthesis of adenosine triphosphate (ATP), the main energy storage in the cell [*Takada et al., 1981*]. Dehydrogenase activity in the studied *Bacillus* strains was the greatest at the incubation temperature 5 °C (0.165 mg/(mL·day)), being on average 0.112 mg/(mL·day) and 0.005 mg/(mL·day) at 22 °C and 45 °C, respectively (Table 3). The optimal temperatures for dehydrogenase catalytical activity are 5 and 22 °C, being typical of cold-adapted bacteria.

All strains exhibited exolipase activity (Table 3), which was the greatest at 5 °C, up to 0.800–3.042 μmol/(mL·min), and especially high in *B. cereus* 630 (3.04 μmol/(mL·min)), *B. cereus* 875 (2.66 μmol/(mL·min)) and *B. simplex* 948P-1 (2.62 μmol/(mL·min)). Lipase activity varied from 0.360 to 2.000 μmol/(mL·min) at 22 °C and from 0.001 to 0.014 μmol/(mL·min) at 45 °C. The strains of *B. cereus* species showed high lipase activity at 5 and 22 °C. Exolipase production depended on strain species: *B. cereus* were more active at 5 °C while the activity of *B. megaterium* and *B. subtilis* was high at 22 °C.

The ability of psychrophilic and psychrotolerant bacteria to produce cold-active amylase at low temperatures provides cell membrane fluidity thus maintaining the ionic and molecular compositions of cells [*Hébraud and Potier, 1999*].

The most rapid production of α-amylase was observed in *B. cereus* 875 (0.39 U/(mL·min)), *B. megaterium* 629 (0.32 U/(mL·min)), and *B. cereus* 1257 (0.24 U/(mL·min)) at 5 °C, and in *B. cereus* 630 (0.28 U/(mL·min)) and *B. cereus* 875 (0.24 U/(mL·min)) at 22 °C (Table 3) but decreased markedly, to 0.02–0.06 U/(mL·min), at 45 °C. *Kuddus et al.* [*2011, 2012*] reported similar temperature dependence of α-amylase activity in bacterial strains from cold habitats: it was the highest within 20 °C, decreased at warmer temperatures, and disappeared at 50 °C.

Protease activity was estimated during the late stationary phase proceeding from the knowledge gained previously through studies of proteolytic bacterial growth [*Dube et al., 2001*]. Proteases in psychrophilic and psychrotolerant bacteria are remarkable by high catalytical activity in cold and temperate conditions, higher than in mesophilic microorganisms. High protease activity is a common adaptation mechanism of microbiota to cold environments by maintaining membrane fluidity and participation of cold-specific proteases in recognition and destruction of denaturated proteins which can lead to cell death [*Potier et al., 1990*].

High protease activity was noted in *B. megaterium* 206 (0.34 U/(mL·30 min)), *Bacillus* sp. 3M (0.25 U/(mL·30 min)) and *B. subtilis* 948P (0.22 U/(mL·30 min)) grown at 22 °C. At 5 °C, it was the highest in *B. simplex* 948P-1 (0.20 U/(mL·30 min)), *B. cereus* 875 (0.17 U/(mL·30 min)) and *B. cereus* 1562 (0.16 U/(mL·30 min)) (Table 3). Compared with the published evidence on protease activity of microorganisms from cold habitats, our results suggest that the temperature optimum for psychrophilic and psychrotolerant bacteria falls within 0 to 30 °C, and is lower than for mesophilic strains. Production of proteases reduces at higher temperatures [*Vazqueza et al., 2004; Struvay and Feller, 2012; Fornbacke and Clarsund, 2013*].

CONCLUSIONS

The reported experimental study shows that temperatures from 5 to 22 °C are optimal for growth of the *Bacillus* genus strains. Therefore, bacteria isolated from permafrost are psychrotolerant and can thrive within a large temperature range. Active synthesis of specific enzymes is a universal mechanism providing adaptation of bacteria to low positive and negative temperatures. Enzymes that exhibit high activity at low positive temperatures can compensate cell metabolism disorders thus allowing bacterial cells to keep growing, though at a slower rate. The experimental data confirm increased enzyme activity in most of the studied *Bacillus* strains at low positive temperatures.

The reported results extend our idea on tolerable temperature limits for growth and enzyme activity of permafrost microbiota, as well as on potential uses of cold-adapted microbes in applied biotechnological research.

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