Chapter 11
Interaction of Microorganisms with Nanomaterials as a Basis for Creation of High-Efficiency Biotechnological Preparations

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Contents

11.1 Introduction

In natural conditions, microorganisms function in close interaction with solid materials, including interaction with nanoparticles of natural minerals, which are significant components of the soil (Costerton et al. 1985; Zehnder et al. 1996; Mishra and Kumar 2009; Nannipieri et al. 2017). A relevant role in this interaction is played by particles of silicon dioxide and clay minerals. The properties of these particles may change significantly as the soil particle size decreases to the nanoscale (Zhang et al. 2017). Clay minerals have considerable effects on the physical, chemical, and biological processes of soils, as well as the physiological and biochemical activities of microorganisms in different taxonomic groups (Kurdish 2010; Zhang et al. 2018).

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The properties of the surface are important factors, determining the interaction of microorganisms and nanoparticles. Nanoparticles of different origins are characterized by a large specific surface area with a negative charge and different functional groups thereon, which determine the specificities of the interactions between microorganisms and these particles considerably. Regardless of the total negative charge, the surface of cells and particles of solid materials may contain positively charged and hydrophobic sites, which promote contact interaction between microorganisms and particles of solid materials and other objects (Rutter et al. 1984). It has been demonstrated that nanoparticles of natural soil montmorillonite and kaolinite are efficient sorbents for organic compounds (He et al. 2015).

The interaction of different kinds of microorganisms and particles of clay minerals promotes the survival of cells in soil conditions (van Veen et al. 1997), including the impact of protozoa (Heijnen et al. 1988), coexistence with which affects both the number of microorganisms and the content of biologically active substances in the environment (Pogorelova et al. 2012).

11.2 Influences of Nanomaterials on Growth, Activity, and Viability of Microorganisms

By use of electronic microscopy and microelectrophoresis, it was established that after the introduction of silica nanoparticles (Aerosil A-300) or nanoparticles of clay minerals (bentonite, montmorillonite, or palygorskite) into a suspension of bacteria, they start to interact; as a result, the cells get covered with particles of these materials (Fig. 11.1) (Gordienko et al. 1993).

The interaction between bacteria and nanomaterials has considerable impacts on the physiological and biochemical activities of microbial populations. It was demonstrated by us that during cultivation of the bacteria *Methylomonas rubra* 15sh

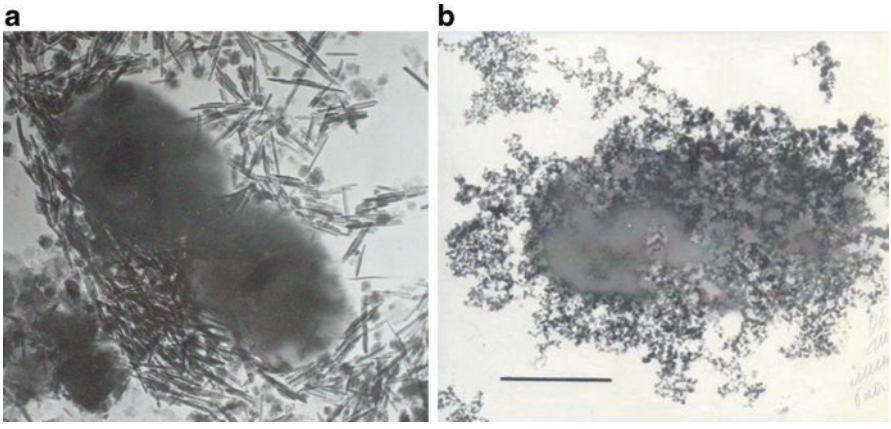

Fig. 11.1 Cells of *Methylomonas rubra* 15sh after interaction with nanoparticles of palygorskite (**a**) and silicon dioxide (**b**)

or *Methylococcus capsulatus* BCB-874 in a culture medium containing 50–200 mg/l of nanoparticles of silicon dioxide or palygorskite, the methane-oxidizing and growth activities of these microorganisms increased considerably (Kurdish and Kigel 1997).

Introduction of silica nanoparticles into the culture medium was accompanied by an increase in the growth activity of the yeast *Saccharomyces cerevisiae* (race XII). The maximal accumulation of biomass in conditions of periodic cultivation of the yeast was observed with a 0.05–0.1% content of this nanomaterial in the medium (Fig. 11.2). An increase in its concentration resulted in a decrease in the biomass gain, and at a content exceeding 0.3% the level of biomass accumulation was lower than that seen in the control.

A further increase in the content of silica nanoparticles was accompanied by more vividly expressed inhibition of yeast growth (Kurdish et al. 1991a). A similar regularity regarding the impact of these nanoparticles was observed during cultivation of the yeast *Candida tropicalis* K-41 (Tsimberg et al. 1991).

A similar effect of these particles was observed during cultivation of *Azotobacter* bacteria. For instance, during cultivation of *Azotobacter chroococcum* 20 in Ashby's medium containing 0.05% silica nanoparticles, the number of these bacteria doubled in comparison with the control. The kinematic viscosity of the culture liquid was also increased by accumulation of a considerable amount of polysaccharide therein (Table 11.1).

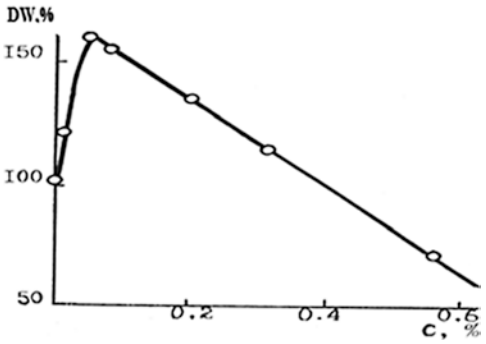

Fig. 11.2 Dependence of biomass accumulation (calculated per total dry weight (*DW*) of yeast biomass) of *S. cerevisiae* on the content (*C*) of silica nanoparticles in the medium

Table 11.1 Effects of silicon dioxide (*SD*) nanoparticles and their modified forms on the growth of *Azotobacter chroococcum* 20 in Ashby's medium and on the kinematic viscosity of the suspension

	Viable bacteria		Kinematic viscosity of the suspension	
Type of nanoparticles	CFU · 10^8/ml	% versus control	mm/cm^2	% versus control
No nanoparticles	0.24 ± 0.05	100.0	6.1 ± 0.1	100.0
SD nanoparticles	0.48 ± 0.06	200.0	17.3 ± 0.3	283.6
SD-AE nanoparticles	0.55 ± 0.06	229.3	20.4 ± 0.4	334.4
SD-Al nanoparticles	0.97 ± 0.17	404.0	84.7 ± 5.0	1388.5

The content of nanoparticles in the medium was 0.05%
CFU Colony-forming units, *SD-AE* silicon dioxide particles modified with aminoethoxy groups, *SD-Al* silicon dioxide particles modified with aluminum oxide

In a medium containing 0.05% silica dioxide nanoparticles modified with aminoethoxy groups (SD-AE) or aluminum oxide (SD-Al), these indicators were higher (Kurdish et al. 1993a). Cultivation of *A. chroococcum* 20 or *A. vinelandii* 56 in a medium containing 0.05% silicon dioxide or alumo-Aerosil resulted in considerable increases in thiamine (vitamin B_1) and pyridoxine (vitamin B_6) content therein (Titova et al. 1994).

Cultivation of *Agrobacterium radiobacter* 10 or *A. radiobacter* 204 in a medium containing 0.05% silica nanoparticles was accompanied by a 32% increase in the growth of bacteria in comparison with the control. The numbers of *A. radiobacter* 10 were increased by 71.5% and 78.6% with addition of 1% montmorillonite or palygorskite nanoparticles, respectively, in comparison with the control. However, cultivation of these bacteria with kaolinite particles resulted in a 28.6% decrease in the number of bacterial cells (Kurdish and Titova 2001) (Table 11.2).

Cultivation of the legume bacteria *Bradyrhizobium japonicum* 634b in a medium with clay mineral particles was also accompanied with a considerable increase in their growth activity (Table 11.3). After 5 days of cultivation in medium without clay minerals, the number of bacteria increased up to 5.0×10^{10} cells/ml. The same index was an order higher in the presence of 1 g/l of nanoparticles of palygorskite, montmorillonite, or bentonite in the medium (Kurdish and Melnykova 2011).

We bred highly active strains of the phosphate-mobilizing bacteria *Bacillus subtilis* IMV B-7023 (Kurdish and Roy 2003) and the nitrogen-fixing bacteria *A. vinelandii* IMV B-7076 (Kurdish and Bega 2006a), promoting the growth and development of plants considerably (Kurdish 2010). It was established that cultivation of *A. vinelandii* IMV B-7076 in Berk's medium containing 0.1–5.0 g/l of silica nanoparticles was accompanied by a considerable increase in the growth activity of these bacteria (Fig. 11.3). With a silica nanoparticle content of 0.5 g/l in

Table 11.2 Effects of clay mineral nanoparticles on the growth of *Agrobacterium radiobacter* 10

Type of nanoparticles	Viable bacterial cells, %
No nanoparticles (control)	100.0 ± 6.2
Palygorskite	178.6 ± 5.4
Montmorillonite	171.5 ± 16.0
Kaolinite	128.6 ± 11.3

The mineral content of the medium was 1 g/l

Table 11.3 Effects of clay mineral nanoparticles on the growth of *Bradyrhizobium japonicum* 634b

Type of nanoparticles	Viable bacterial cells/ml of culture medium		
	At 1 day	At 3 days	At 5 days
No nanoparticles (control)	$(8.4 \pm 1.6) \times 10^6$	$(3.1 \pm 0.2) \times 10^8$	$(5.0 \pm 0.4) \times 10^{10}$
Palygorskite	$(2.4 \pm 0.4) \times 10^6$	$(2.3 \pm 0.1) \times 10^9$	$(8.2 \pm 0.2) \times 10^{11}$
Montmorillonite	$(3.1 \pm 0.6) \times 10^6$	$(5.3 \pm 1.0) \times 10^9$	$(2.5 \pm 0.6) \times 10^{11}$
Bentonite	$(1.5 \pm 0.1) \times 10^6$	$(1.2 \pm 0.2) \times 10^9$	$(6.3 \pm 0.6) \times 10^{11}$

The mineral content of the medium was 1 g/l

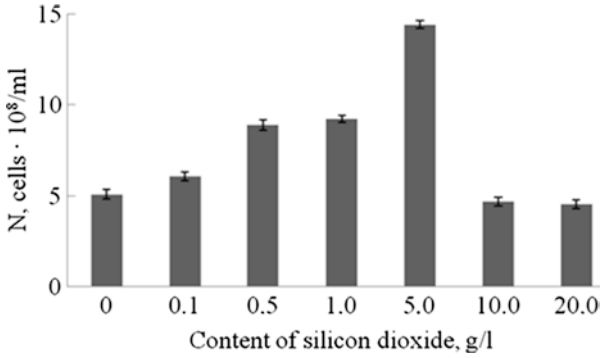

Fig. 11.3 Numbers of viable cells of *Azotobacter vinelandii* IMV B-7076 during cultivation in Berk's medium with silica nanoparticles

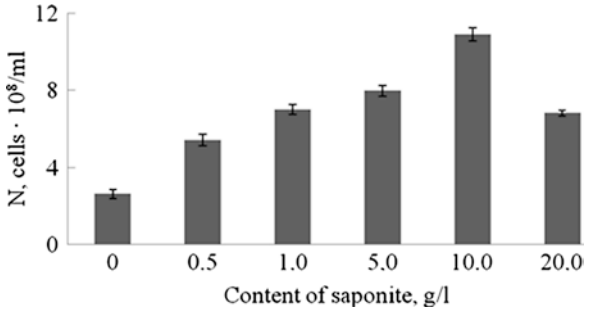

Fig. 11.4 Dependence of the numbers of viable cells of *Azotobacter vinelandii* IMV B-7076 on the content of saponite particles in the culture medium

the culture medium, the number of bacteria therein increased by 71% in comparison with the control. The maximal growth activity was obtained after addition of 5 g/l of silica nanoparticles to the culture medium (Chobotarov et al. 2010a). In these conditions, the number of *A. vinelandii* IMV B-7076 cells was 2.8 times that in the control conditions after 48 h of cultivation in the culture medium (Fig. 11.3). At the same time, the viscosity of the culture medium increased 55 times. Data in the scientific literature have confirmed that silica nanoparticles increase the content of bacteria in soil conditions considerably, promoting germination of corn seeds (Karunakaran et al. 2012).

Saponite particles had a stimulating effect on the growth of *A. vinelandii* IMV B-7076. As a result of introduction of 0.5 g/l of this mineral into the culture medium, the number of bacteria almost doubled in comparison with the control (Fig. 11.4). An increase in the content of saponite particles led to enhanced growth activity of these microorganisms. Their greatest increased occurred at a concentration of 10.0 g/l of this mineral in the medium. Under such conditions the number of cells in the experimental variants was almost four times that in the control. A further

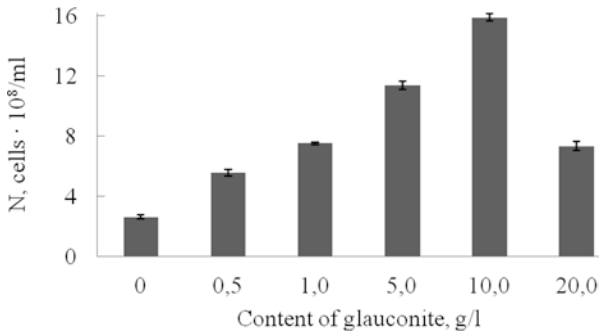

Fig. 11.5 Dependence of the numbers of viable cells of *Azotobacter vinelandii* IMV B-7076 on the content of glauconite particles in the culture medium

increase in the content of saponite in the medium was accompanied by a decrease in its stimulating effect on the growth of these bacteria (Chobotarov et al. 2010a).

Addition of glauconite to the culture medium significant affected the growth of the nitrogen-fixing bacteria *A. vinelandii* IMV B-7076. After introduction of 0.5 g/l of glauconite particles, the number of these bacteria doubled in comparison with the control (Chobotarov et al. 2010a). The greatest growth of bacteria was observed during their cultivation in a medium containing 5.0 or 10.0 g/l of these mineral particles (Fig. 11.5). Under these conditions the numbers of *Azotobacter* were 4.3 and 6.0 times that in the control conditions, respectively. A further increase in the content of this mineral in the medium was accompanied by a decrease in its stimulating effect on bacterial growth (Chobotarov et al. 2010a) (Fig. 11.5).

Cultivation of this *Azotobacter* strain in a medium with phosphorite particles also stimulated the growth activity of these bacteria. The maximal stimulating effect was observed during cultivation of the bacteria in a medium containing 5 g/l of mineral particles. Under these conditions, the number of viable cells was five times that seen in the control conditions (Chobotarov et al. 2010a).

It was established that cultivation of *B. subtilis* IMV B-7023 in a medium containing saponite particles had an obvious stimulating effect on the growth activity of these bacteria. At a content of 0.5 g/l of saponite particles in the medium, the number of these bacterial cells increased by 80% in comparison with the control. The stimulating effect of saponite was enhanced with an increase in its content in the medium. The maximal growth of cells was observed in media containing 5 g/l and 10 g/l of saponite; under these conditions the numbers of bacteria were 2.3 and 2.4 times that in the control medium, respectively (Chobotarov et al. 2010b, 2013). Therefore, introduction of nanoparticles of silicon dioxide, saponite, glauconite, and phosphorite into the culture medium considerably increased the growth activity of *A. vinelandii* IMV B-7076 and *B. subtilis* IMV B-7023.

It was demonstrated by us that *B. subtilis* IMV B-7023 is capable of absorbing phosphorus from both its organic (Roy et al. 2001) and inorganic compounds, including the poorly soluble calcium phosphate $Ca_3(PO_4)_2$ (Bulavenko et al. 2000). A considerable influence on growth was the presence of particles of clay minerals in a medium containing $Ca_3(PO_4)_2$ as the single source of phosphorus (Kurdish and

Bega 2006a). Their stimulating effect on the growth of these bacteria depended on the size of the particles of these minerals. Cultivation of *B. subtilis* IMV B-7023 in a medium containing 0.2% free-flowing montmorillonite increased the number of these bacteria by 67%. A more noticeable increase in bacterial growth activity was observed during cultivation of this strain with nanoparticles of this mineral obtained after its ultrasonic processing. In these conditions, the number of bacteria was increased by 72% in comparison with the control. The maximal values of growth activity were obtained during cultivation of *B. subtilis* IMV B-7023 in a medium containing 0.5% montmorillonite particles. A further increase in the content of this mineral in the medium was accompanied by a decrease in the growth activity of the bacteria (Kurdish and Bega 2006a).

The presence of palygorskite particles had a more obvious stimulating effect on the growth activity of *B. subtilis* IMV B-7023. Cultivation of bacteria in a medium containing 0.2% free-flowing palygorskite particles resulted in an increase in the number of bacteria by 43%. In the presence of nanoparticles of this mineral the number of cells was enhanced by 56% in comparison with the control. The most noticeable stimulating effect on the growth activity of the bacilli was observed during cultivation in a medium containing 1% palygorskite nanoparticles. In this variant, the number of bacteria was increased by 263% in comparison with the control. The increase in the content of nanoparticles in the cultivation medium was accompanied by a decrease in their stimulating effect on bacterial growth (Kurdish and Bega 2006a).

It was demonstrated that the growth activity of methanotrophic bacteria is due to the concentration of the substrates and the ratios between the bacterial biomass and the specific concentrations of the substrates (Kurdish et al. 1988, 1990). To assess the influence of mineral nanoparticles on the growth activity of *B. subtilis* IMV B-7023, we investigated the features of its growth by cultivating it for 20 h in a culture medium containing calcium phosphate and montmorillonite particles (1%) and different initial densities of the population of bacteria. In the control variant, the cultivation of *B. subtilis* was conducted without the presence of the clay mineral.

The results of the study (Table 11.4) demonstrated that when *B. subtilis* was cultivated in a medium not containing particles of the clay mineral, the highest specific rate of bacterial growth (0.29 h^{-1}) was obtained in the presence of the lowest

Table 11.4 Dependence of the growth of *Bacillus subtilis* IMV B-7023 on montmorillonite content in the culture medium and the initial density of the bacterial population

Montmorillonite content, %	Viable bacterial cells/ml of culture medium		Specific growth rate, μ	Culture medium pH
	At the start of cultivation	At the end of cultivation		
0	$(8.42 \pm 0.68) \cdot 10^5$	$(2.87 \pm 0.41) \cdot 10^8$	0.29	5.30
0	$(8.61 \pm 0.40) \cdot 10^6$	$(2.83 \pm 0.32) \cdot 10^8$	0.19	5.28
0	$(4.13 \pm 0.54) \cdot 10^7$	$(3.71 \pm 0.78) \cdot 10^8$	0.11	5.40
1	$(8.42 \pm 0.68) \cdot 10^5$	$(3.53 \pm 0.46) \cdot 10^8$	0.30	5.08
1	$(8.61 \pm 0.40) \cdot 10^6$	$(5.26 \pm 0.75) \cdot 10^8$	0.21	5.07
1	$(4.13 \pm 0.54) \cdot 10^7$	$(6.54 \pm 0.60) \cdot 10^8$	0.14	5.08

The cultivation period was 20 h

initial density of the bacterial population (8.42×10^5 cells per milliliter) (Kurdish and Bega 2006a). With an increase in the latter, the specific rate of growth of the microorganisms decreased considerably in the medium with an initial density of 4.13×10^7 bacteria per milliliter; the specific rate of growth amounted to 0.11 h^{-1} (Table 11.4).

During cultivation of *B. subtilis* IMV B-7023 in a medium with montmorillonite particles, the specific rates of growth were higher than in the absence of these particles. However, a regularity concerning the dependence of the growth of the bacteria on the initial content of biomass, similar to the aforementioned regularity, was observed in this experimental variant as well (Kurdish and Bega 2006a). Therefore, the obtained results demonstrate that under such conditions the growth rate of *B. subtilis* is determined by the concentrations of both nutrient substrates and mineral particles in the medium and their ratios to the number of bacteria—that is, their specific concentrations.

A stimulating effect of the interaction between microorganisms and nanomaterials on the physiological activity of microbial populations may be conditioned by a number of factors. One of the mechanisms of the stimulating effect that nanoparticles of the studied minerals have on the growth activity of microorganisms may be found in the enhanced mass transfer of oxygen at their introduction into the medium used for cultivation of the bacteria. Stirring of the medium with mineral nanoparticles results in formation of highly turbulent microzones around the aforementioned particles, which are capable of conditioning the increase in the mass transfer of oxygen (Kurdish 2001). It was demonstrated by us that introduction of 1% and 4% powdered palygorskite into the medium resulted in increases in the mass transfer of oxygen by 6% and 15.5%, respectively. The introduction of nanoparticles of this mineral and silicon dioxide caused a noticeable effect on this indicator. At a content of 1% palygorskite in the medium, the mass transfer of oxygen therein increased by 16.9% (Kurdish 2001).

As one of the relevant factors for aerobic microorganism functioning is the supply of oxygen to them, an impact of this mechanism is possible. At the same time, it should be noted that an increase in the content of mineral nanoparticles by >4% resulted in an increase in the medium viscosity, accompanied by a decrease in the mass transfer of oxygen. This may cause a negative effect on the growth activity of aerobic microorganisms (Kurdish 2001).

Taking the above into consideration, we were interested in studying the impact of different concentrations of oxygen on the growth of these bacteria. It was demonstrated that with an increase of oxygen mass transfer into the medium from 0.41 to 1.83 g $O_2 \cdot l \cdot h^{-1}$, the growth activity of *B. subtilis* IMV B-7023 was boosted, whereas that of *A. chroococcum* 21 was decreased.

To study the effect of mineral nanoparticles on the growth activity of these bacteria with different values of mass transfer of oxygen, they were cultivated in hermetic vials with artificial gas mixtures (Kisten et al. 2006). After 13 h of incubation at a content of about 3% O_2 in the gas phase, the number of bacteria that grew was 57.1% greater in the medium with palygorskite than in the control medium (with no palygorskite). The increase in the oxygen content up to 6% resulted in the increase in the number of bacteria by 148.3%, and at 20.7% O_2 by 487.5% compared against

Table 11.5 Effects of the clay mineral palygorskite on accumulation of *Azotobacter chroococcum* 21 cells at different concentrations of O_2 in the gas phase

	Incubation in culture medium				
	For 0 h	For 13 h		For 24 h	
Palygorskite content, mass %	O_2, vol. %	O_2, vol. %	Viable bacterial cells/ml	O_2, vol. %	Viable bacterial cells/ml
0	20.7	10.6	$(1.6 \pm 0.5) \cdot 10^8$	8.6	$(2.1 \pm 0.8) \cdot 10^8$
1.0	20.7	6.7	$(9.4 \pm 0.7) \cdot 10^8$	4.0	$(1.2 \pm 0.2) \cdot 10^9$
0	12.6	5.7	$(2.0 \pm 0.3) \cdot 10^8$	5.2	$(2.7 \pm 1.2) \cdot 10^8$
1.0	12.6	3.8	$(9.5 \pm 1.2) \cdot 10^8$	1.8	$(1.3 \pm 0.2) \cdot 10^9$
0	6.9	3.3	$(2.9 \pm 0.5) \cdot 10^8$	2.3	$(3.1 \pm 0.6) \cdot 10^8$
1.0	6.8	2.0	$(7.2 \pm 0.8) \cdot 10^8$	1.2	$(8.9 \pm 1.6) \cdot 10^8$
0	3.5	1.4	$(2.1 \pm 0.4) \cdot 10^8$	1.4	$(2.8 \pm 0.4) \cdot 10^8$
1.0	3.8	1.1	$(3.3 \pm 0.3) \cdot 10^8$	1.0	$(5.3 \pm 1.3) \cdot 10^8$

vol. Volume

the control (Table 11.5). Cultivation of *A. chroococcum* in the aforementioned conditions for 24 h resulted in rapid decreases in the concentrations of glucose and phosphate and in the content of O_2 in the gas phase of the hermetic vials. Regardless of this fact, the clay mineral particles stimulated the growth of these bacteria.

Therefore, the obtained results demonstrated that the most traceable stimulating effect of palygorskite particles on the growth activity of *Azotobacter* was shown at high concentrations of oxygen. It was shown that during cultivation of *A. chroococcum* 21 bacteria in Ashby's medium, palygorskite protects cells from the toxic effect of high concentrations of oxygen to some degree.

A relevant impact on the growth of microorganisms may be caused by ion exchange processes occurring in the medium upon the introduction of nanomaterials. It is known that protein may be adsorbed on the surface of silica nanoparticles and other mineral particles (Kisten et al. 2006). It was demonstrated by us that introduction of saponite particles into a *B. subtilis* IMV B-7023 cultivation medium led to an evident increase in the concentrations of the cations Mg^{2+}, Ca^{2+}, Na^+, and K^+ (by between 63 and 191 mg/l, respectively) (Chobotarov et al. 2010b). Therefore, the increase in the physiological activity of microorganisms upon their interaction with nanomaterials of different natures may be conditioned by the impacts of many factors, and determining their actions is a relevant prerequisite for accomplishment of biotechnological tasks.

It was demonstrated that interaction of the bacteria *M. rubra* 15sh, *Pseudomonas aureofaciens* UKM-111, *A. radiobacter* 204, and other microorganisms with nanoparticles of the clay minerals montmorillonite, palygorskite, and bentonite had a considerable stimulating effect on the viability of cells in long-term storage and during exposure to increased temperature (Gerasymenko and Kurdish 2015; Kurdish et al. 1993a, b, 1999; Kurdish and Antonyuk 1999). For instance, incubation of an *A. radiobacter* 204 suspension at 50 °C for 15 min (as a control treatment) was accompanied by a 40% decrease in the number of viable cells. However, in the case of previous introduction of 1% palygorskite particles into such a suspension, the number of viable cells decreased by only 20% (Kurdish and Antonyuk 1999).

A similar effect on the viability of *B. japonicum* 634b was caused by particles of this mineral (Kurdish et al. 1999). When suspensions of these bacteria were incubated at 45 °C for 15 min in the absence of clay mineral nanoparticles, 32.4–40.6% of cells were viable (Table 11.6). Introduction of montmorillonite nanoparticles into the suspension of this strain was accompanied by an increase in the resistance of the cells to the effect of supraoptimal temperatures. For instance, in the presence of 1 g/l of montmorillonite particles, the percentage of viable cells after warming of the suspension was increased to 69%, and in the presence of 10 g/l of these nanoparticles, the percentage was increased to 82.7%. A similar effect on the influence of supraoptimal temperatures on *B. japonicum* 634b was observed with introduction of palygorskite nanoparticles into the suspension (Table 11.6).

Therefore, it was demonstrated that the interaction of the investigated species of bacteria and clay mineral particles considerably increases the resistance of cells to the effect of increased temperatures on them.

It was shown (Kurdish and Antonyuk 1999) that the interaction of *A. chroococcum* 20 and clay mineral particles also considerably increases their resistance to supraoptimal temperatures. In the absence of minerals, the cells of these bacteria are very sensitive to the effect of higher temperatures. After incubation of the bacteria at 45 °C for 10 min without clay minerals, only 12.7% of cells in the suspension were viable, but after interaction between these microorganisms and palygorskite (0.5%), the proportion of viable cells was increased to 34.5% (Table 11.7). Montmorillonite particles had even a more evident effect on the viability of *Azotobacter* (Kurdish and Antonyuk 1999).

It should be noted that this effect of mineral particles was enhanced with an increase in the content of montmorillonite in the medium. At a concentration of 10 g/l the proportion of viable bacteria was increased to 68%—more than three

Table 11.6 Effects of clay mineral nanoparticles on resistance of *Bradyrhizobium japonicum* 634b to warming at 45 °C for 15 min

	Viable bacterial cells, % versus control (accepted as 100%)	
Clay mineral particle content, g/l	With montmorillonite	With palygorskite
0	40.6 ± 3.9	40.6 ± 3.9
1.0	69.0 ± 4.2	67.1
5.0	64.8 ± 5.1	ND
10.0	82.7 ± 8.1	ND

ND Not determined

Table 11.7 Effects of clay mineral nanoparticles on resistance of *Azotobacter chroococcum* 20 to warming at 45 °C for 10 min

	Viable bacterial cells, % versus control (accepted as 100%)	
Clay mineral particle content, g/l	With montmorillonite	With palygorskite
0	12.7 ± 1.4	12.7 ± 0.8
0.1	28.5 ± 1.8	28.4 ± 1.8
0.5	59.4 ± 3.7	34.5 ± 2.1
1.0	68.0 ± 4.2	32.4 ± 2.0

times that in the control conditions. Therefore, the interaction between *Azotobacter* and clay mineral particles increases not only the growth, nitrogen-fixing activity, and synthesis of B vitamins by these microorganisms, but also the viability of these bacteria with exposure to supraoptimal temperatures. The technology of using nanoparticles of natural minerals, developed by us to promote survival of bacteria in case of their exposure to extreme environmental factors, is a promising approach for improving the storage of collection strains of microorganisms and for creating new forms of bacterial preparations (Kurdish 2001).

Taking into consideration the protective impact of clay mineral particles on the survival of microorganisms, we developed biotechnology to enhance the yield of viable bacteria in preparations (Kurdish and Titova 2000) produced by the method of spray drying (Gordienko et al. 1990). For this purpose, 10 g/l of palygorskite particles was introduced into the suspension of bacteria. The mixture was stirred, and after 15 min of interaction between the cells and the mineral particles, we introduced a protective medium (dry skim milk), and then the composite underwent further spray drying. This method allowed the yield of viable *Streptococcus faecium* cells and other species of bacteria to be increased by >60% during manufacturing of their preparations by the aforementioned technology in comparison with the control treatment (without mineral particles, but with dry milk) (Gordienko et al. 1990; Kurdish et al. 1991b). The aforementioned biotechnology was implemented in the production of the Litosyl preparation at the Kiev Pharmaceutical Drugs Plant.

11.3 Influences of Nanoparticles on Physiological and Biochemical Activities of Microorganisms

It is known that the physiological and biochemical activities of microorganisms depend on their cultivation conditions, which determine the accumulation of a number of metabolites in the medium that are capable of affecting other components of the biota (Niste et al. 2013). We demonstrated that the energy potential of cells was increased by interaction between bacteria and mineral nanoparticles. During cultivation of *B. subtilis* IMV B-7023 in a culture medium containing 5 g/l of exfoliated vermiculite, the dehydrogenase activity of the bacteria was increased by 34% in comparison with the control (Gerasymenko and Kurdish 2015).

During cultivation of these bacteria in a medium with addition of 0.5 g/l of silica nanoparticles, the dehydrogenase activity of *B. subtilis* IMV B-7023 was increased by only 6–7% in comparison with the control. Silica nanoparticles had an insignificant effect on the dehydrogenase activity of *A. vinelandii* IMV B-7076. However, during cultivation of this strain in a medium containing vermiculite nanoparticles, the indices of the dehydrogenase activity of *Azotobacter* were higher; when 5.0 g/l of vermiculite nanoparticles were added to the medium, the dehydrogenase activity was 40% higher in comparison with the control (Fig. 11.6). Therefore, the introduction of vermiculite nanoparticles into the medium was accompanied by an increase in the dehydrogenase activity of the investigated bacteria (Gerasymenko and Kurdish 2015; Kurdish et al. 2014).

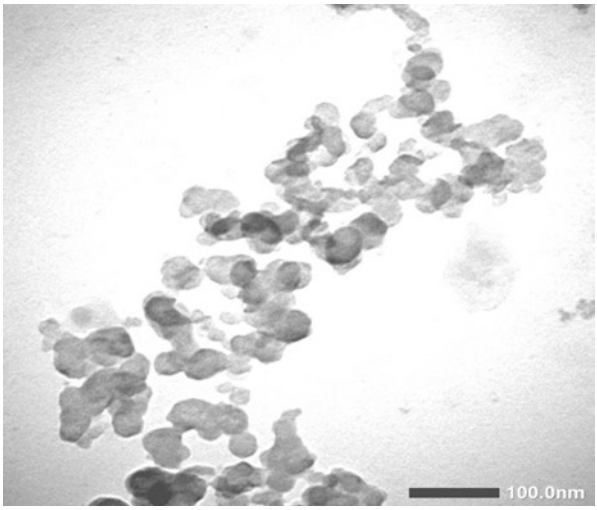

Fig. 11.6 Vermiculite particles after processing with an ultrasonic disintegrator

A relevant property of microorganisms (as components of different ecosystems and strains) that is promising for biotechnological applications, including plant production, is their capability to synthesize biologically active substances that can affect other representatives of the biota, such as plants, by influencing their growth and development and protecting them against the impacts of negative factors.

It was demonstrated by us that during cultivation of the phosphate-mobilizing bacteria *B. subtilis* IMV B-7023 in Menkina medium with calcium glycerophosphate and glucose, accumulation of various amino acids (arginine, isoleucine, valine, proline, and phenylalanine) and organic acids occurred (Table 11.8), with acetic acid (up to 32.5 μg/ml) and pelargonic acid (up to 6 μg/ml) being the most abundant (Tsercovniak et al. 2009b; Skorochod et al. 2013).

During cultivation of *B. subtilis* in a medium with addition of 5.0 g/l of titanium dioxide nanoparticles, the total content of amino acids in the culture medium increased by 27.7% in comparison with the control (Table 11.8). Under these conditions the content of phenylalanine was 7.85 μg/ml, histidine 2.63 μg/ml, and valine 1.59 μg/ml. Insignificant concentrations of glutamic acid, asparagine, and methionine were also accumulated in the culture medium. No traces of isoleucine was found.

The total amounts of free amino acids accumulated in the culture media of *B. subtilis* IMV B-7023 with addition of particles of the natural minerals glauconite or saponite were 6.80 and 3.46 μg/ml, respectively—1.7 and 3.4 times lower, respectively, than the total amino acid content accumulated with the control treatment (Table 11.8). These findings may have been conditioned by the adsorption of a certain part of these substances on the surface of the mineral nanoparticles.

Cultivation of *A. vinelandii* IMV B-7076 in Ashby's medium was accompanied by accumulation of an insignificant amount of amino acids therein, the total amount of which was up to 2.78 μg/ml (Table 11.9). An evident stimulating impact on the

Table 11.8 Free amino acid content in a culture medium containing *Bacillus subtilis* IMV B-7023, depending on the type of mineral particle content

Amino acid	Amino acid content, μg/ml			
	With no mineral particles (control)	With titanium dioxide[a]	With glauconite[a]	With saponite[a]
Alanine	ND	ND	ND	0.29 ± 0.02
Arginine	2.90 ± 0.08	ND	ND	ND
Asparagine	ND	0.37 ± 0.08	ND	ND
Valine	1.04 ± 0.04	1.59 ± 0.04	1.00 ± 0.01	1.25 ± 0.08
Histidine	ND	2.63 ± 0.08	2.77 ± 0.03	ND
Glutamic acid	ND	1.73 ± 0.01	ND	ND
Isoleucine	4.11 ± 0.05	ND	0.69 ± 0.08	ND
Methionine	ND	0.90 ± 0.01	ND	0.37 ± 0.09
Proline	2.12 ± 0.06	ND	ND	ND
Serine	ND	ND	0.67 ± 0.04	ND
Tryptophan	ND	ND	0.30 ± 0.06	ND
Phenylalanine	1.63 ± 0.03	7.85 ± 0.03	1.37 ± 0.05	1.55 ± 0.07
Total amino acid content	11.80 ± 0.08	15.07 ± 0.08	6.80 ± 0.05	3.46 ± 0.09

ND Not determined
[a]The content of mineral particles in the medium was 5.0 g/l
[b]$p < 0.01$

Table 11.9 Free amino acid content in a culture medium containing *Azotobacter vinelandii* IMV B-7076, depending on the type of mineral particle content

Amino acid	Amino acid content, μg/ml			
	With no mineral particles (control)	With titanium dioxide[a]	With glauconite[a]	With saponite[a]
Alanine	ND	ND	0.13 ± 0.02	ND
Arginine	ND	ND	6.98 ± 0.05	ND
Asparagine	ND	1.58 ± 0.06	ND	0.32 ± 0.01
Valine	0.58 ± 0.08	ND	0.56 ± 0.04	1.42 ± 0.09
Histidine	ND	1.23 ± 0.09	1.91 ± 0.03	ND
Glycine	ND	ND	0.14 ± 0.03	ND
Glutamic acid	ND	ND	1.15 ± 0.02	ND
Isoleucine	ND	ND	ND	0.34 ± 0.08
Methionine	ND	ND	ND	0.74 ± 0.07
Proline	ND	ND	ND	9.55 ± 0.10
Tryptophan	ND	ND	0.57 ± 0.01	0.86 ± 0.06
Phenylalanine	2.18 ± 0.08	ND	3.16 ± 0.02	3.16 ± 0.10
Cysteine	ND	ND	ND	0.29 ± 0.09
Total amino acid content	2.76 ± 0.08	2.81 ± 0.07	14.58 ± 0.03	16.67 ± 0.10

ND Not determined
[a]The content of mineral particles in the medium was 5.0 g/l
[b]$p < 0.01$

synthesis of amino acids by these bacteria was caused by their cultivation in a medium containing nanoparticles of some natural minerals. During cultivation of these bacteria in a medium with glauconite particles, eight amino acids were found, the total content of which was up to 14.4 μg/ml. Arginine, phenylalanine, and histidine were the most abundant. In a medium containing 5 g/l of saponite, the total accumulation of amino acids exceeded 16 μg/ml (which was 6 times greater than in the control) (Table 11.9).

The highest accumulated concentrations were noted for proline (9.55 μg/ml), phenylalanine (3.16 μg/ml), and valine (1.42 μg/ml) (Kurdish et al. 2014; Chobotarov 2015). Therefore, particles of the natural minerals saponite and glauconite have an evident stimulating effect on accumulation of free amino acids in a culture medium of *A. vinelandii* IMV B-7076.

One of the most important properties of bacteria is their ability to stimulate and improve plant growth and development by producing phytohormones. About 95% of soil microorganisms can produce different hormonal compounds that regulate physiological and biochemical reactions in plants (including stress responses) and play an essential role in plant growth and development (Davies 2004).

Auxins have a considerable impact on the growth and development of plants. They accelerate the process of root formation and affect the processes of photosynthesis, growth, tropism, blossoming, and fruit bearing of plants. As a rule, L-tryptophan should be added to the medium for synthesis of indole acetic acid (IAA), the source of which in soil conditions may be found in root excretions (Shakirova 2001).

It has been established that *B. subtilis* IMV B-7023 and *A. vinelandii* IMV B-7076 bacteria are capable of synthesizing a number of substances of a phytohormonal nature. Cultivation of *A. vinelandii* IMV B-7076 in a medium with L-tryptophan was accompanied by accumulation of 140 ng/ml of free IAA therein, and about 160 ng/ml of this auxin was in a bound state (Tsercovniak et al. 2009a). During cultivation of these bacteria without L-tryptophan, much lower concentrations of this phytohormone were accumulated.

The total amount of IAA in the medium containing *A. vinelandii* IMV B-7076 amounted to 67.1 ng/ml. After cultivation of these bacteria in a medium with nanoparticles of silicon dioxide or vermiculite, the total amount of this phytohormone in the medium was much smaller (Chobotarov et al. 2017a, b).

Cultivation of *B. subtilis* IMV B-7023 in a glucose–mineral medium was accompanied by accumulation of 46 ng/ml of IAA therein. However, during cultivation of these bacteria in a medium with silica nanoparticles, the total accumulation of this phytohormone was almost doubled. There was a particular increase in the content of IAA during cultivation of these bacteria in a medium with vermiculite: 4.6 times the amount observed in the absence of these particles (Chobotarov et al. 2017a).

Among phytohormones, abscisic acid (ABA) plays an important role in plants. ABA accumulates in plants upon their exposure to stress factors and is involved in bud differentiation, fruit development, and formation of additional roots (Kulaeva 1973). The data obtained have revealed that *A. vinelandii* IMV B-7076 accumulates ABA in its culture medium (Table 11.10). The ABA content not associated with other organic compounds was 8.1 ng/ml, while the content of its bound form was 21.5 ng/ml (Chobotarov et al. 2017a, b).

Table 11.10 Effects of nanoparticles on the ability of *A. vinelandii* IMV B-7076 and *B. subtilis* IMV B-7023 to accumulate abscisic acid (*ABA*) in the culture medium

Type of nanoparticles	ABA form	ABA accumulation, ng/ml	
		By *A. vinelandii* IMV B-7076	By *B. subtilis* IMV B-7023
No nanoparticles (control)	Free	8.1 ± 0.4	41.4 ± 1.1
	Bound	21.5 ± 1.0	ND
Nano-SiO_2	Free	10.8 ± 0.5	10.5 ± 0.5
	Bound	9.8 ± 0.5	14.7 ± 0.7
Vermiculite	Free	48.6 ± 1.4	65.0 ± 1.8
	Bound	20.3 ± 1.0	4.2 ± 0.2

ND Not determined

Table 11.11 Effects of nanoparticles on accumulation of cytokinins in a culture medium of *A. vinelandii* IMV B-7076

Phytohormone	Phytohormone content, ng/ml		
	With no nanoparticles (control)	With silica[a]	With vermiculite[a]
Zeatin	53.9 ± 1.7	129.2 ± 1.5	53.6 ± 1.7
Zeatin riboside	49.2 ± 1.5	176.8 ± 1.3	113.6 ± 1.7
Zeatin glycoside	104.3 ± 1.2	107.4 ± 1.3	83.3 ± 1.2

[a]The content of mineral particles in the medium was 5.0 g/l

Addition of 5.0 g/l of nano-SiO_2 stimulated biosynthesis of the free form of ABA in a culture medium of *A. vinelandii* IMV B-7076 by 1.3 times, whereas the amount of the bound form of ABA decreased rapidly. With addition of 5.0 g/l of vermiculite nanoparticles to a culture medium of *A. vinelandii* IMV B-7076, a substantial increase in ABA synthesis was observed, with the total ABA content being 2.3 times that seen in the control conditions. The most noticeable increase, resulting in an ABA content six times that seen in the control conditions, was observed for the free ABA form not associated with other organic compounds.

B. subtilis IMV B-7023 is also capable of ABA accumulation (41.4 ng/ml) in a culture medium. The total amount of ABA in a culture medium of *B. subtilis* IMV B-7023 was lower with addition of SiO_2 nanoparticles than in the control; the content of the free and bound ABA forms was 10.5 ng/ml and 14.7 ng/ml, respectively. Addition of vermiculite to a culture medium of *B. subtilis* IMV B-7023 increased synthesis of the free ABA form to 65.0 ng/ml, which was 1.4 times that observed with the control, whereas the concentration of its bound forms was 3.5 times lower than in the variants with addition of nano-SiO_2 (Chobotarov et al. 2017a).

Cytokinins play an important role in regulation of plant growth and development (Shakirova 2001; Giron et al. 2013). It was established that *A. vinelandii* IMV B-7076 can accumulate different compounds of a cytokinin nature in its culture medium (Table 11.11). The amounts of zeatin, zeatin riboside, and zeatin glycoside were 53.9, 49.2, and 104.3 ng/ml, respectively (Chobotarov et al. 2017a, b). Cultivation of these bacteria in a medium with SiO_2 nanoparticles increased accumulation of zeatin by 2.4 times, zeatin riboside by 3.6 times, and zeatin glycoside by 3% (Table 11.11).

Addition of vermiculite particles to a culture medium of *A. vinelandii* stimulated synthesis of zeatin riboside, resulting in a content 2.3 times that seen in the control, but did not change the content of zeatin and actually reduced the content of zeatin glycoside by 20% (Table 11.11).

B. subtilis IMV B-7023 bacteria are also capable of cytokinin production (Chobotarov et al. 2017a). Addition of vermiculite and silica dioxide particles to a culture medium of *B. subtilis* resulted in a significant increase in cytokinin production (Kurdish et al. 2014). Thus, *A. vinelandii* IMV B-7076 and *B. subtilis* IMV B-7023 bacteria are capable of plant hormone synthesis. Cultivation of bacteria in a culture medium with silica or vermiculite nanomaterials promotes accumulation of phytohormones.

A relevant role among those of the biologically active compounds of cells, including microorganisms, is attributed to metabolites of a phenolic nature. They participate in initiation of interactions between bacteria and plants (Long 2001), and they are important components of the cell, capable of protecting them from reactive oxygen intermediates (Skorochod and Kurdish 2018) and phytopathogenic microorganisms (Kiprushkina and Kolodyaznaya 2014). It was demonstrated by us that during cultivation of *B. subtilis* IMV B-7023 in Menkina medium, these bacteria accumulated a number of phenolic compounds, with phenylacetic and 4-hydroxyphenyl-acetic acids being the most abundant (Tserkovniak and Kurdish 2009). It was established that 4-hydroxyphenyl-acetic acid is capable of stimulating the development of plants and inhibiting the growth of the phytopathogenic micromycetes *Fusarium culmorum*, *Fusarium solani*, *Alternaria alternata*, whose zones of growth inhibition were 21–31 mm.

It was shown that accumulation of phenolic compounds in a culture medium of *A. vinelandii* IMV B-7076 depends on the type of carbon source and its concentration in the medium. The greatest concentration of phenolic compounds in the culture medium (223 μg/ml) was observed during cultivation of the bacteria with 30 g/l of glucose (Ocheretyanko et al. 2016). It was established that during cultivation of the strain *A. vinelandii* IMV B-7076 with 0.05–0.1 g/l of bentonite nanoparticles, the content of phenolic compounds in the culture medium exceeded that in the control by 2–16%. However, when *A. vinelandii* IMV B-7076 was cultivated with silica nanoparticles, an increase in phenolic compound content in the culture medium was observed only in variants containing 0.05–0.1 g/l of nano-SiO_2. After introduction of 0.5 g/l of silica nanoparticles into the culture medium, the phenolic compound content decreased (Skorochod and Kurdish 2018).

It is known that different microbial metabolites may protect cells against reactive oxygen species (Skorochod and Kurdish 2014). Among these are enzymes (catalase, peroxidase, and superoxide dismutase (SOD)) (Labas et al. 2010), low molecular weight antioxidants of the thiol redox system (e.g., glutathione) (Filomeni et al. 2002), phenolic antioxidants, and other bioactive substances (Shtarkman et al. 2008). Phenolic antioxidants effectively inhibit peroxide, alkoxy radicals, hydroxyl radicals, superoxide anion radicals, and singlet oxygen (He et al. 2015). In addition, some phenolic compounds in microorganisms selectively inhibit the functioning of individual species of phytopathogenic micromycetes (Kohen and Nyska 2002).

It was established that *B. subtilis* IMV B-7023 and *A. vinelandii* IMV B-7076 bacteria are characterized by a high degree of antioxidant and antiradical protection. Cultivation of *B. subtilis* IMV B-7023 in a medium containing low concentrations of silica nanoparticles was accompanied by activation of antioxidant protection of the cells, whereas at a silica nanoparticle content of 1 g/l, antioxidant activity was decreased by 11.8%, hydroxyl radical scavenging was decreased by 17.6%, and oxidation activity was increased by 26.9% (Skorochod et al. 2016). A considerable impact on these indices was made by cultivation of *B. subtilis* IMV B-7023 in a medium with vermiculite nanoparticles (Skorochod and Kurdish 2013).

The first link in the chain of protection of living cells from reactive oxygen intermediates is found in such enzymes as catalase, peroxidase, and SOD (Labas et al. 2010). We studied the dependence of the activity of these enzymes in *B. subtilis* IMV B-7023 and *A. vinelandii* IMV B-7076 bacteria on the content of mineral particles in the culture medium.

It was shown that with addition of 1.5 or 2.5 g/l of vermiculite nanoparticles into the culture medium, the peroxidase activity of *B. subtilis* IMV B-7023 tripled. However, with a higher content of vermiculite nanoparticles (5 g/l), the extracellular peroxidase activity was lower (Table 11.12).

It was established that during cultivation of *B. subtilis* IMV B-7023 in a medium containing 0.05 g/l of silica nanoparticles, the extracellular peroxidase activity of the bacteria increased by 43.8% and the intracellular activity by 74.2% (Skorochod and Kurdish 2013). These indicators increased with further increases in the content of these nanoparticles in the medium to 0.1 and 0.5 g/l. However, the extracellular and intracellular peroxidase activity decreased after addition of 1 g/l of silica nanoparticles to the medium. Silica and vermiculite nanoparticles did not have any substantial effect on the extracellular and intracellular catalase activity of *B. subtilis* IMV B-7023 or on the intracellular peroxidase activity (Skorochod and Kurdish 2013).

Some effect of particles of these natural minerals on SOD activity of *A. vinelandii* IMV B-7076 was observed. We demonstrated that cultivation of these bacteria in a medium containing 1 g/l of bentonite or saponite nanoparticles was accompanied by boosted SOD activity of the bacteria. A more evident increase in this index was observed during cultivation of *Azotobacter* with bentonite particles together with addition of 0.5 mM of manganese ions to the medium. SOD activity was 17.5% higher in this medium than in the medium containing bentonite alone and 31.5% higher than during cultivation without these ions and nanoparticles (Chobotarov et al. 2017a, b). Cultivation of this strain in a medium with both saponite nanoparticles and Mn^{2+} ions had a less considerable impact on SOD activity of these bacteria.

Table 11.12 Effects of vermiculite nanoparticles on antioxidant enzyme activity in a culture medium of *B. subtilis* IMV B-7023

Vermiculite nanoparticle content, g/l	Catalase activity, mmol of H_2O_2/min/mg of protein	Peroxidase activity, mmol of indigo carmine/min/mg of protein
0	6.98 ± 0.12	0.75 ± 0.03
1.5	7.61 ± 0.17	1.92 ± 0.89
2.5	7.69 ± 0.17	2.09 ± 0.95
5.0	7.79 ± 0.18	1.61 ± 0.65

It was established that cultivation of *A. vinelandii* IMV B-7076 in a medium containing 0.25 mM of Fe^{2+} ions stimulated the SOD activity of this strain to some degree. However, an increase in the content of these cations in the medium was accompanied by inhibition of SOD activity. The obtained results demonstrate a considerable effect of saponite and bentonite nanoparticles on the SOD activity of the investigated bacteria.

Therefore, *A. vinelandii* IMV B-7076 and *B. subtilis* IMV B-7023 synthesize a number of organic acids, amino acids, phenol compounds, and phytohormones that are capable of improving the growth and development of plants. Cultivation of these strains in culture media containing nanoparticles of natural materials stimulates the growth of these bacteria and their accumulation of biologically active substances in the media.

11.4 Impacts of Interaction Between Bacteria and Nanomaterials on Chemotaxis

Along with physical and chemical factors, a significant role in the interaction between microorganisms and other surfaces, including nanomaterials, is attributed to mobility of cells (van der Mei et al. 2001), which is affected by hydrodynamic forces, sedimentation (Marshall 1985), the sensor properties of cells, and their ability to have taxis. Taxis of microorganisms it has a remarkable role in the process of their interaction with other objects, including solid surfaces (Begonia and Kremer 1994). Cells may react to different factors: a number of chemical factors, light, the pH of the medium, magnetic fields, etc. (Begonia and Kremer 1994; Bashan and Holguin 1997). Through chemotaxis, bacteria may move to places where they interact with solid particles, especially at the surface of plant roots, as considerable quantities of plant metabolites are released into the space near the roots, which may be attractive for bacteria (Kravchenko et al. 2003).

The bacterial examples *B. japonicum* 634b, *B. subtilis* IMV B-7023, and *A. vinelandii* IMV B-7076 were used by us to demonstrate that bacteria demonstrate chemotaxis regarding a wide range of carbohydrates, amino acids, and organic acids (Kurdish et al. 2001, 2010; Chuiko and Kurdish 2004, 2017; Chuiko et al. 2006). We investigated the effects of a number of factors, including nanoparticles of different natures, on the chemotaxis properties of these microorganisms. It was demonstrated that interaction of bacteria with nanoparticles of silicon dioxide or clay minerals promotes the mobility of the cells considerably. However, the chemotaxis of bacilli and nodule bacteria was decreased (Chuiko and Kurdish 2004; Chuiko et al. 2006). After interaction with montmorillonite in a concentration of 0.2 g/l, the mobility of *B. japonicum* 634b increased by 25% (Chuiko and Kurdish 2004).

A similar increase in mobility was observed in the strain *B. japonicum* 604k. After introduction of montmorillonite in a concentration of 0.1 g/l into a suspension of these bacterial cells, the quantitative indicators of their mobility increased

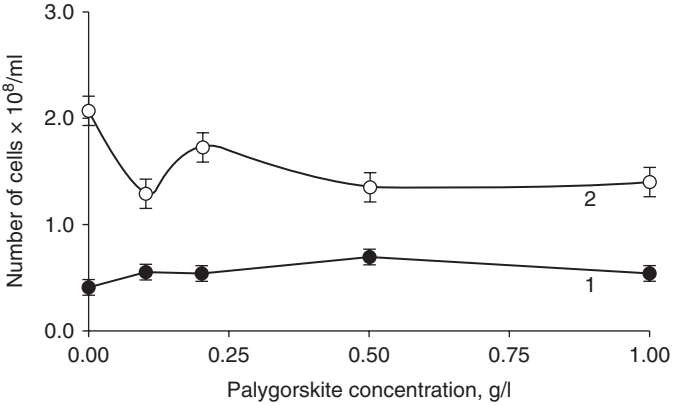

Fig. 11.7 Effects of palygorskite on the mobility and chemotaxis properties of *Bradyrhizobium japonicum* 634b. *1* Number of bacterial cells in capillaries containing phosphate buffer, *2* number of bacterial cells in capillaries containing 5.6×10^{-2} M of glucose

by 27%, and at a mineral concentration of 0.5 g/l, their mobility increased by 105% (Chuiko and Kurdish 2004).

Interaction of bacteria and palygorskite particles also increased the mobility of the investigated strains of *B. japonicum*, but somewhat less than their interaction with montmorillonite (Chuiko and Kurdish 2004). The chemotaxis of bacteria decreased at the content of palygorskite nanoparticles. With addition of 0.1 g/l of these nanoparticles to a suspension of *B. japonicum* 634b, the chemotaxis of the bacteria decreased by 38% (Fig. 11.7).

Similar dependence of the mobility of cells on the content of silica and clay mineral nanoparticles in their suspension was also observed in *B. subtilis* IMV B-7023 and *A. vinelandii* IMV B-7076 (Kurdish et al. 2010; Chuiko and Kurdish 2017). Introduction of 0.05–0.2 g/l of saponite particles into the phosphate buffer did not affect the mobility and chemotaxis of *B. subtilis* IMV B-7023. However, with an increase in the saponite content to 0.5–1.0 g/l, the mobility of these bacteria increased by 121–124% and their chemotaxis to glucose decreased by 2.2–3.7 times. A stimulating impact on the mobility of these bacteria was caused by introduction of 0.05–0.5 g/l of silicon dioxide into the phosphate buffer. In this case, the content of bacilli in capillaries containing the phosphate buffer was 11–46% higher than that in the absence of the nanoparticles. The chemotaxis of these bacteria was decreased the most by a content of 1 g/l of these nanoparticles.

A decrease in the chemotaxis of bacilli was also observed during their contact with the polysaccharide complex of *Azotobacter*, which was adsorbed on the cell surfaces of *B. subtilis* IMV B-7023; this was accompanied by an increase in the zeta potential of the bacilli from −35.8 mV to −46 mV, which is notable for *Azotobacter* cells (Chuiko et al. 2013).

During interaction of *A. vinelandii* IMV B-7076 with saponite particles (0.1–1.0 g/l), 5–22% more cells entered capillaries with phosphate buffer, which demonstrated stimulation of chaotic mobility of these bacteria by the aforementioned

particles. However, the number of bacterial cells in capillaries containing glucose along with mineral particles differed from that seen in the previous variant considerably (Chuiko and Kurdish 2017). This may have been influenced by the fact that during interaction of nanoparticles and the surface of the bacilli, these particles block chemotaxis receptors, whereas in *A. vinelandii* IMV B-7076 they may be under the polysaccharide layer, protecting these receptors from interaction with nanoparticles and blocking their function. Therefore, the interaction of the investigated bacteria with silica, montmorillonite, and palygorskite particles promoted an increase in the mobility of their cells. This effect may have been influenced by an increase in the energetic metabolism of the bacteria during their interaction with the investigated nanomaterials. This phenomenon could promote distribution of these microorganisms in natural conditions.

It was demonstrated by us that the increase in the mobility of *B. subtilis* IMV B-7023 and *A. vinelandii* IMV B-7076 with the impact of some factors correlates with their enhanced adhesion to plant roots (Kurdish et al. 2008a, b, c). It was established that bacteria that were selected in the phase of their logarithmic growth (24 h) had the highest adhesive activity of *Azotobacter* to the roots of Konkurent cucumber plants. An evident decrease in the chemotaxis of bacteria was observed in the stationary phase of growth (after 72 h of cultivation). The bacterial cells adhered to the surface of the plant roots in much smaller quantities, and after their cultivation for 96 h the number of adhered cells on this surface was only a tenth of that seen after cultivation of the bacteria for 24 h. A considerable decrease in the adhesion of these bacteria was influenced by high indices of the negative charge of their surface and the loss of cell mobility during their long-term cultivation (Kurdish et al. 2008a, b, c).

The decrease in the chemotaxis of bacteria upon their interaction with nanoparticles of natural minerals demonstrates the possibility of blocking chemotaxis receptors on the surface of bacteria by the investigated nanoparticles and the polysaccharide complex of *Azotobacter* (Chuiko and Kurdish 2004, 2017; Chuiko et al. 2006, 2013). Therefore, the results of these investigations demonstrate that interaction between the investigated species of bacteria and nanoparticles of many minerals is accompanied by an increase in the mobility of cells and a reduction in their chemotaxis.

11.5 Interaction of Plant Growth–Promoting Rhizobacteria and Natural Mineral Nanomaterials as a Basis for Highly Efficient Preparations for Plant Production

Taking into consideration the stretch properties of clay minerals, their stimulating effects on the physiological and biochemical activity of bacteria, the protective effect of nanoparticles on the viability of cells, their antioxidant properties, and the interaction of our selected highly active strains of the nitrogen-fixing bacteria *A. vinelandii* IMV B-7076 and the phosphate-mobilizing bacteria *B. subtilis* IMV

Table 11.13 Numbers of viable cells of *A. vinelandii* IMV B-7076 and *B. subtilis* IMV B-7023 in the granulated complex bacterial preparation Azogran during storage at different temperatures (Kurdish and Bega 2006b)

Storage period, months	Viable bacterial cells/g			
	At room temperature		At 4 °C	
	A. vinelandii IMV B-7076	*B. subtilis* IMV B-7023	*A. vinelandii* IMV B-7076	*B. subtilis* IMV B-7023
0	$(7.1 \pm 0.3) \cdot 10^8$	$(3.8 \pm 0.2) \cdot 10^8$	$(7.0 \pm 0.3) \cdot 10^8$	$(3.5 \pm 0.2) \cdot 10^8$
3	$(4.6 \pm 0.3) \cdot 10^8$	$(3.4 \pm 0.2) \cdot 10^8$	$(5.1 \pm 0.4) \cdot 10^8$	$(3.5 \pm 0.3) \cdot 10^8$
6	$(1.8 \pm 0.3) \cdot 10^8$	$(2.8 \pm 0.1) \cdot 10^8$	$(2.1 \pm 0.2) \cdot 10^8$	$(3.4 \pm 0.1) \cdot 10^8$

B-7023 with particles of bentonite, we created a granulated complex bacterial preparation, named Azogran, for use in plant production (Kurdish 2010), containing $>10^8$ viable cells of each species of bacteria per gram of the preparation. This preparation is stable during long-term storage. It is registered in Ukraine and used in plant production. The number of bacteria in the granulated preparation is somewhat dependent on the storage temperature (Kurdish 2010). After 6 months of storage at room temperature, the number of viable cells of *A. vinelandii* IMV B-7076 therein was >25% of the initial number, and after 6 months of storage at 4°C, it was 30% of the initial number. Under these conditions of storage of the preparation, the number of viable *B. subtilis* IMV B-7023 cells was decreased to some degree (Table 11.13).

This preparation improves nitrogen and phosphorus nutrition of plants, and it stimulates their growth and development through synthesis of many biologically active substances by the bacteria (including substances of a phytohormonal nature) and the ability to inhibit plant damage by phytopathogens and phytophages. It was established that *B. subtilis* IMV B-7023 is an antagonist of many strains of phytopathogenic bacteria and micromycetes (Roy et al. 2005). This strain inhibits the growth of phytopathogenic bacteria (*Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *atrofaciens*, *Erwinia carotovora*, *Clavibacter michiganensis*, and *Agrobacterium tumefaciens*) and many species of phytopathogenic micromycetes (*Fusarium graminearum*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium sambucinum*, *Bipolaris sorokiniana*, *Alteromonas alternate*, and *Gliocladium roseum*) (Roy et al. 2005). *B. subtilis* IMV B-7023 bacteria inhibit spreading of the agent of bacterial cancer of tomatoes considerably (Roy et al. 2012).

It was observed that presowing treatment of Beliy Naliv tomato seeds with a suspension of the phytopathogen *Clavibacter michiganensis* subsp. *michiganensis* was accompanied by a reduction in plant growth. However, combined inoculation of the seeds with this phytopathogen and *B. subtilis* IMV B-7023 enhanced their germination by 14.6% and plant growth by 16–18%; the development of bacterial cancer in these plants was not determined. However, when control plants were treated with this phytopathogen (their seeds were treated with water), they developed classic symptoms of bacterial cancer (Roy et al. 2012).

A relevant factor in the efficiency of use of the complex bacterial preparation Azogran in plant production is its capability to inhibit distribution of both phytopathogens and phytophages in agroecosystems. It was established by us that epiphytical treatment of plants using a suspension of the bacteria that are components of the preparation decreases distribution of many species of phytophages in agroecosystems (Roy et al. 2014; Zubko and Kurdish 2017) and improves the growth and development of plants considerably. It was determined that treatment of flowering plants (*Coleus* and *Pelargonium*, planted in a greenhouse) with a suspension of *B. subtilis* IMV B-7023 considerably decreased the numbers of greenhouse whitefly (*Trialeurodes vopariorum*) and green peach aphid (*Aulacorthum circumflexus*) phytophages by 50–70%. It was shown that subsequent treatment of the plants with a suspension of the studied bacteria provided effective biocontrol of phytophages in greenhouse conditions (Zubko and Kurdish 2017).

Taking into consideration the fact that the interaction of *B. subtilis* IMV B-7023 and *A. vinelandii* IMV B-7076 with vermiculite particles stimulates the physiological and biochemical activities of these bacteria considerably and improves their survival during long-term storage (Table 11.14), we also created a free-flowing form of the complex bacterial preparation Azogran on the basis of the interaction between these strains and particles of exfoliated vermiculite, which is convenient for bacterization of plant seeds (Kurdish and Roy 2014).

Creation of a free-flowing complex bacterial preparation requires optimization of the culture conditions used for production. We optimized the composition of culture media for cultivation of the individual bacterial monocultures and mixed bacterial cultures that are components of the complex preparation. It was established that the best medium for growth of the mixed bacterial culture is a liquid culture medium with the following composition: treacle 30.0 g/l, corn steep extract 2.0 g/l, $K_2HPO_4 \cdot 3H_2O$ 0.25 g/l, KH_2PO_4 0.25 g/l, $MgSO_4 \cdot 7H_2O$ 0.3 g/l, NaCl 0.3 g/l, and $CaCO_3$ 3.0 g/l (pH 7.0–7.2). Cultivation of mixed cultures of *A. vinelandii* IMV B-7076 and *B. subtilis* IMV B-7023 in this medium for 24 h yields a culture liquid with more than 10^9 cells of each strain per milliliter (Table 11.14) (Kurdish et al. 2015).

The process used for manufacturing this free-flowing complex bacterial preparation based on the highly active strains *A. vinelandii* IMV B-7076 and *B. subtilis* IMV B-7023 was optimized. Because the bacilli were characterized as having a higher specific growth rate than *A. vinelandii* IMV B-7076, the inoculation of the

Table 11.14 Numbers of *A. vinelandii* IMV B-7076 and *B. subtilis* IMV B-7023 cells during incubation in a preparation containing powdered vermiculite

	Viable bacterial cells/g		
Bacterial species	At 0 days	At 1 day	At 2 days
A. vinelandii *B. subtilis*	$(4.0 \pm 0.1) \cdot 10^7$ $(1.4 \pm 0.1) \cdot 10^7$	$(1.9 \pm 0.1) \cdot 10^9$ $(3.8 \pm 0.2) \cdot 10^9$	$(1.7 \pm 0.1) \cdot 10^9$ $1.0 \pm 0.1) \cdot 10^{10}$
A. vinelandii *B. subtilis*	$(3.7 \pm 0.2) \cdot 10^7$ $(1.9 \pm 0.1) \cdot 10^6$	$(1.6 \pm 0.1) \cdot 10^9$ $(1.1 \pm 0.1) \cdot 10^9$	$(1.8 \pm 0.1) \cdot 10^9$ $(3.7 \pm 0.4) \cdot 10^9$
A. vinelandii *B. subtilis*	$(3.8 \pm 0.3) \cdot 10^7$ $(1.2 \pm 0.1) \cdot 10^5$	$(2.6 \pm 0.2) \cdot 10^9$ $(3.0 \pm 0.1) \cdot 10^8$	$(2.1 \pm 0.1) \cdot 10^9$ $(6.0 \pm 0.2) \cdot 10^8$

vermiculite-mixed suspension of bacteria in the optimized culture medium amounted to about 10^7 colony-forming units (CFU) per milliliter of *A. vinelandii* IMV B-7076 and 10^6 CFU/ml of *B. subtilis* IMV B-7023 in a 3:1 mass ratio to this carrier. Thus, incubation with vermiculite for 24 h at 28 °C ensured high quality of the resulting complex bacterial preparation containing >10^9 CFU of *A. vinelandii* IMV B-7076 and *B. subtilis* IMV B-7023 per gram (Table 11.14) (Kurdish et al. 2015).

It was established by us that the obtained free-flowing preparation Azogran based on the bacteria *A. vinelandii* IMV B-7076 and *B. subtilis* IMV B-7023 could be stored well at room temperature (20–23 °C) for a long time (Table 11.15).

During storage for 6 months, both strains of these bacteria were defined in the preparation to the ninth power. After 1 year of storage, the number of *B. subtilis* cells exceeded 10^9 cells/g, while the number of *A. vinelandii* had decreased to 6.6×10^8 cells/g. Thus, it was established that Azogran, a granulated and free-flowing complex bacterial preparation, was characterized by compositional stability during long-term storage (for up to 6 months).

The complex bacterial preparation Azogran causes a more evident stimulating effect on the growth and development of plants than use of monoculture preparations (Kurdish 2010). Introduction of the complex bacterial preparation Azogran into agroecosystems considerably improves the growth and development of lawn grass, many species of decorative plants (*Chlorophytum*, dragon tree, jade tree, boxwood, and *Thuja*), flowering plants (*Begonia*, roses, and others), and seedlings and young plants of pine and fir trees. It also enhances yields of technical crops, cereals, and vegetables by 16–37% (Kurdish 2010; Kurdish et al. 2008a, b, c; Skorokhod et al. 2012).

Introduction of one granule of the complex bacterial preparation Azogran into the root zone of Ilius roses increased the number of inflorescence shoots on the plants by 45% (Kurdish 2010; Kurdish et al. 2008a, b, c). However, its stimulating effect decreased with introduction of two granules of this preparation (Table 11.16). A less evident impact was made by introduction of one granule of the Azogran preparation into the root zone of Grand Prix roses (Table 11.16). In this case, the number of inflorescence shoots increased by 22.6%, but after introduction of two granules, the stimulating action of the preparation, again, decreased.

Table 11.15 Dependence of the numbers of viable cells of *B. subtilis* IMV B-7023 and *A. vinelandii* IMV B-7076 in a free-flowing complex bacterial preparation on the storage duration at 20–23 °C

Storage period, months	Bacterial species	Viable bacterial cells/g
0	*A. vinelandii* *B. subtilis*	$(4.3 \pm 0.2) \times 10^9$ $(6.2 \pm 0.1) \times 10^9$
2	*A. vinelandii* *B. subtilis*	$(2.5 \pm 0.2) \times 10^9$ $(6.0 \pm 0.4) \times 10^9$
6	*A. vinelandii* *B. subtilis*	$(2.0 \pm 0.2) \times 10^9$ $(2.4 \pm 0.3) \times 10^9$
12	*A. vinelandii* *B. subtilis*	$(6.6 \pm 0.2) \times 10^8$ $(1.4 \pm 0.05) \times 10^9$

Table 11.16 Effects of a granulated complex bacterial preparation (Azogran) on the numbers of inflorescence shoots in Ilius and Grand Prix roses

	Cut inflorescence shoots			
	Ilius		Grand Prix	
Preparation	*N*	%	*N*	%
No preparation (control)	471	100.0	610	100.0
One granule	683	145	748	122.6
Two granules	587	125	658	107.9
$HIP_{0.5}$	94.5	–	97.4	–

Table 11.17 Effects of granulated bacterial preparations of *A. vinelandii* and *B. subtilis* IMV B-7023 on the yield of Chervona Strila tomato species

		Yield gain	
Preparation	Yield, kg	kg	%
No preparation (control)	2556.5	–	100.0
A. vinelandii 56	3037.1	480.5	118.8
A. vinelandii IMV B-7976	3264.5	708.0	127.7
A. vinelandii 56 + *B. subtilis* IMV B-7023	3351.6	795.1	131.1
A. vinelandii IMV B-7076 + *B. subtilis* IMV B-7023	3502.4	945.9	137.0

It was established that introduction of one granule of the complex granulated preparation Azogran into the root zone of young coniferous plants was accompanied by intensification of their growth. The most evident impact of this preparation was observed in *Thuja*, European spruce, and juniper plants. After 4.5 months of growth, *Thuja* plants treated with the preparation were 17% taller, young European spruce trees were 15.0% taller, and juniper plants were 22% taller than their respective controls (Chuiko et al. 2010).

It was demonstrated that introduction of two granules (0.5 g) of Azogran preparation into the root zone of Olexandria sugar beet improved the yield of seeds considerably. After introduction of two granules of a preparation based on one strain of *A. vinelandii* IMV B-7076, the yield of seeds increased by 21.3%. After introduction of two granules of a complex bacterial preparation based on both these bacteria and *B. subtilis*, this index increased by 37.8% (Kurdish et al. 2005).

It was established that introduction of two granules of a monoculture (the mass of one granule is 0.2 g) based on the nitrogen-fixing bacteria *A. vinelandii* 56 during planting of Chervona Strila tomato species increased the yield of the plants by 18.8% (Table 11.17). With use of a monoculture based on *A. vinelandii* IMV B-7076, the yield of the plants increased by 27.7%. However, the most marked stimulating impact on the investigated plants was seen with introduction of the complex bacterial preparation Azogran containing strains of *A. vinelandii* IMV B-7076 and *B. subtilis* IMV B-7023. In this case, the yield of tomatoes increased by 37% (Table 11.17).

It was determined that processing of Tsarivna winter wheat seeds with the free-flowing complex bacterial preparation Azogran increased the grain yield up to 0.57–0.67 t/ha and also increased the content of crude protein and fiber in the grain up to 0.6–1.0% and 1.1–1.3%, respectively. Moreover, the occurrences of root rot lesions and *Septoria* leaf spot on the wheat were decreased significantly (Kurdish et al. 2015). Seed treatment of Nabat spring barley with the free-flowing complex bacterial preparation increased the yield of the grain up to 0.35–0.43 t/ha, increased its content of crude protein up to 0.4–0.6%, and reduced the occurrence of dark brown spot lesions on the plant leaves (Kurdish et al. 2014, 2015).

Thus, the technology for creation of the free-flowing complex bacterial preparation Azogran has been developed for crop growing. This preparation is stable during storage and convenient for use in cereal agroecosystems. Positive impacts of application of this free-flowing complex bacterial preparation to crop seeds of winter wheat and spring barley have been shown. The preparation promotes a significantly increased yield and improves the quality of grain.

It has been determined that in the rhizosphere soil of cereals whose seeds have been treated with the complex bacterial preparation, significant changes are observed in microbial biocenosis, with increases in the total number of bacteria and in the content of oligotrophic bacteria, phosphate-mobilizing bacteria, and other physiologically trophic groups of microorganisms (Kurdish et al. 2014, 2015).

One of the relevant factors that determine the efficiency of using the complex bacterial preparation Azogran in plant production is its capability to inhibit distribution of both phytopathogens and phytophages in agroecosystems. It was established by us that epiphytic treatment of plants, using a suspension of these bacteria, decreases distribution of many species of phytophages in agroecosystems (Kurdish et al. 2008a, b, c; Skorokhod et al. 2012) and improves the growth and development of the plants greatly.

11.6 Conclusion and Future Prospects

It has been demonstrated that the interaction of many species of microorganisms with nanomaterials of different natures has an evident stimulating effect on the physiological and biochemical activity of microbial populations, protecting them from the effects of negative environmental factors. Interactions of *Azotobacter vinelandii* IMV B-7076 and *Bacillus subtilis* IMV B-7023 with bentonite and vermiculite particles have been used as the basis for creation of a granulated and free-flowing complex bacterial preparation, Azogran, for use in plant production. This preparation improves the growth and development of decorative, flowering, and other plants considerably, and increases the yield of technical crops, cereals, and vegetables by 16–37%. The obtained results may be used as the basis for creation of novel biotechnologies.

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