ISSN 0026-2617, Microbiology, 2013, Vol. 82, No. 3, pp. 306–315. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © A.V. Shcherbakov, A. V. Bragina, E.Yu. Kuzmina, Christian Berg, A. N. Muntyan, N.M. Makarova, N.V. Malfanova, Massimiliano Cardinale, Gabriela Berg, V.K. Chebota, I.A. Tikhonovich, 2013, published in Mikrobiologiya, 2013, Vol. 82, No. 3, pp. 312–322.

EXPERIMENTAL ARTICLES

Endophytic Bacteria of *Sphagnum* Mosses as Promising Objects of Agricultural Microbiology[1]

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Abstract—*Sphagnum* mosses serve as a unique habitat for microorganisms, which play an important role both for the host plants and the peatland ecosystems. The aim of the present study was to isolate endophytic bacteria from the tissues of *Sphagnum* mosses and to screen them for strains promising for further application in agricultural microbiology. About 50 samples of *Sphagnum fallax* (H. Klinggr.) H. Klinggr. and *Sphagnum magellanicum* Brid. were collected in the Austrian Alps and the Lenindgrad Region of Russia in 2009–2010. Endophytic bacteria were detected inside the moss plants using fluorescent in situ hybridization (FISH) followed by confocal laser scanning microscopy (CLSM). Altogether, 283 isolates were obtained by cultivation on the nutrient media. Examination of the isolates for the antagonistic activity revealed that more than 50% of them could suppress the growth of phytopathogenic and toxigenic fungi. More than 30% of isolates showed some antagonistic activity against microbial phytopathogens. The isolated strains could colonize crops and promote their growth. Molecular-genetic identification coupled with physiological/biochemical characterization showed that the dominant endophytic groups belonged to the genera *Burkholderia*, Pseudomonas, Flavobacterium, *Serratia* and *Collimonas*. The isolated endophytes were shown to be promising objects for the development of effective growth-promoting and protective microbiological preparations to be used in agriculture.

Keywords: *Sphagnum* mosses, endophytic bacteria, biocontrol, plant growth promotion

DOI: 10.1134/S0026261713030107

INTRODUCTION

A large amount of information about bacteria associated with higher vascular plants has been accumulated in the scientific literature. These bacteria can promote the growth and development of the plants by synthesizing plant hormones and vitamins, fixing molecular nitrogen and suppressing the development of bacterial and fungal diseases. Collectively referred to as plant growth-promoting rhizobacteria (PGPR) [1], this group includes soil microorganisms actively colonizing the rhizosphere and the rhizoplane and promoting plant growth. Endophytic bacteria are also rather well-known, having been found in the tissues of major agricultural crops such as rice [2, 3], corn [4, 5], cotton [4, 6], potato [7, 8], sugar cane [8, 9] etc.

However, until recently little has been known about endophytic heterotrophic PGP-bacteria associated with *Sphagnum* mosses. To note, in the present paper we use the classical definition of the term "endophytes", applying it to bacteria inhabiting the internal tissues of healthy plants without harming the host [10, 11]. In our opinion, the specific structure of the internal tissues of *Sphagnum mosses*, which consist of both live chlorophyllose cells and dead hyaline ones, is not a reason to reject this definition.

Berg and colleagues [13–15] have recently shown that green parts of *Sphagnum* plants serve as unique habitats of such bacteria, which play an important role both for the host plants and the peatland ecosystems. The molecular analysis of microbial communities associated with *Sphagnum* mosses has shown that up to 97% of all the cloned sequences of the 16S rRNA genes could belong to non-culturable forms. The taxonomic position and the proportion of the most numerous microbial groups varied depending on the *Sphagnum* species. Nitrogen-fixing bacteria were the

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Table 1. Geographic sites of *Sphagnum* mosses sampling in Austria and in Russia in 2009–2010

Sampling site	Sampling date	Geographical coordinates	pH and temperature of water in the sampling site, average values	*Sphagnum* species	Strains isolated from the sample, the first three symbols of the abbreviation are given
1. Rootmoos, Austria, Styria	02.09.2009	N 47°41′ E 15°09′	pH 4.3–4.5; 8°C	*S. magellanicum* *S. fallax*	AM1 AF1
2. Waasenmoos, Austria, Salzburg	03.09.2009	N 47°18′ E 12°24′	pH 3.3–3.7; 10°C	*S. magellanicum* *S. fallax*	AM2 AF2
3. Pürgschachen Moor, Austria, Styria	04.09.2009	N 47°34′ E 14°20′	pH 3.9–4.2; 8°C	*S. magellanicum* *S. fallax*	AM3 AF3
4. Voloyarvi Bog, Russia, Leningrad Region, Vsevolozhsk District	03.10.2009	N 60°18′ E 30°48′	pH 4.7–5.0; 5°C	*S. magellanicum* *S. fallax*	RM1 RF1
5. Polesye Bog, Leningrad Region, Vyborg District	19.07.2010	N 60°44′ E 29°04′	pH 4.2–4.6; 15°C	*S. magellanicum* *S. fallax*	RM2 RF2
6. Polevoi Mokh Bog, Leningrad Region, Vyborg District	20.07.2010	N 60°42′ E 29°20′	pH 4.3–4.6; 17°C	*S. magellanicum* *S. fallax*	RM3 RF3
7. Oblozhnyi Mokh Bog, Leningrad Region, Vyborg District	21.07.2010	N 60°35′ E 29°8′	pH 4.3–4.5; 12°C	*S. magellanicum* *S. fallax*	RM4 RF4

most prominent ones, possibly because of their key role under conditions of the lack of mineral nutrition elements for the host plants. A similar symbiosis has been demonstrated for methane-oxidizing microorganisms colonizing the external cortex of stems in *Sphagnum cuspidatum* Hoffm. [16].

The aim of the present study was to isolate endophytic heterotrophic bacteria from *Sphagnum* mosses, to screen them for the presence of beneficial properties and to select the most promising strains for further application in agricultural microbiology.

MATERIALS AND METHODS

Sampling of *Sphagnum* Mosses. Endophytic bacteria were isolated from the gametophytes of two *Sphagnum* species, *Sphagnum fallax* and *S. magellanicum* (species names are given following [17]). These two mosses play a different ecological role in peatland ecosystems but often co-occur in the same biotopes and have a rather broad distribution.

Samples of *Sphagnum* gametophytes were taken during field trips to the Austrian Alps and the Leningrad Region in 2009 and 2010, respectively (Table 1). Sphagnum mosses were identified on the basis of anatomical and morphological characters. In both peatland ecosystems the samples of each species were taken from four sites 50–100 m apart. A bunch of 50–100 plants was taken out of the moss cover and placed in a sterile plastic bag, which was kept cool and was transported to the laboratory on the day of sampling. Geographical coordinates of each site were recorded, the surrounding vegetation was described and the water temperature and pH was measured.

Visualization of *Sphagnum* spp. Microbial Associates: Fluorescent in situ Hybridization (FISH) and Confocal Laser Scanning Microscopy (CLSM). Single *Sphagnum* gametophytes were analyzed. Samples were fixed in 4% paraformaldehyde solution mixed with phosphate buffer in the volumetric proportion of 3 : 1 [18]. For further treatment separate branches of moss plants were placed in sterile 1.5 ml tubes. Fixed samples were hybridized with rRNA-specific oligonucleotide probes following [19]. We used for hybridization an equimolar mixture of universal bacterial samples EUB338, EUBII338, EUBIII338 [20–21] in combination with BET42a and GAM42a probes specific for the representatives of the classes *Betaproteobacteria* and *Gammaproteobacteria* [22]. Formaldehyde concentration in the hybridization buffer was 45% for universal bacterial probes and 15% for group-specific probes; hybridization temperature was 41°C. A nonspecific NONEUB probe was used for the negative control [20]. In the end the samples were mounted on an object slide and covered with a ProLong Gold Antifade reagent (Invitrogen, Germany).

The preparations were studied on the Leica TCS SPE confocal microscope (Leica Mycrosystems, Germany). To detect oligonucleotide probes labelled with 6FAM, Cy3 and Cy5 fluorochromes, lasers with a wave length of 488, 532 and 635 nm were used. Fluorescence was registered in the range of 508-566 nm, 665–607 nm and 657–709 nm, respectively. Three-dimensional reconstruction of the images was performed with the use Imaris 7.0 software (Bitplane, Switzerland).

Isolation of Culturable-dependent Endophytic Bacteria. Endophytic bacteria were isolated from *Sphagnum* gametophytes following the previously described

method [23]. Plated Petri dishes were incubated at 20°C for 5 days and the total number of colonies was counted. Bacteria from morphologically different colonies were plated into new Petri dishes using the exhaustive inoculation method. The cultures were tested for purity and their cultural/morphological characteristics were described. For storage the cultures were incubated in agar slant tubes or frozen at –80°C in 20% glycerine solution.

Screening the Isolated Strains for Antagonistic Activity Against Phytopathogenic Fungi and Bacteria. Fungicidal properties of the isolates were tested on the following strains of phytopathogenic and toxigenic fungi: *Fusarium graminearum, Fusarium culmorum, Fusarium sporotrichioides* (strains from the collection of the All-Russia Institute for Plant Protection (VIZR) kindly provided by Dr. Gagkaeva). Cultures of antagonistic bacteria were grown on R2A liquid medium under stationary conditions at 20°C for 3 days; fungicidal activity was assessed with the use of wells [24] on potato-dextrose agar (Difco, USA). Antibacterial properties were tested on four strains of phytopathogenic bacteria: *Erwinia carotovora* var. *atroseptica* 822, *Pseudomonas fluorescens* 213, *Pseudomonas syringae* 8511, *Clavibacter michiganensis* pv. *sepedonicum* 6028 (strains from the VIZR collection, kindly provided by Dr. Lazarev) with the use of agar blocks [25] on 2% potato agar. Growth inhibition zones were measured after incubation at 28°C in the course of 5 days (for fungi) and 2 days (for bacteria).

Molecular-genetic Identification and Physiological/biochemical Characterization of the Most Promising Strains of Endophytic Bacteria. Bacterial DNA was extracted from pure 24-hour cultures of the bacterial strains under study according to the standard procedure (lysis with lysozyme/proteinase K and SDS followed by phenol-chloroform extraction). A fragment of the 16S rRNA gene was amplified with the use of BD1/FD1 primers [26]. Cleaned fragments of the 16S rRNA gene were sequenced with the primers used for amplification according to the protocol of Beckman Coulter (USA) for the SEQ8000 sequencer with the use of the SEQ Dye Terminator Cycle Sequencing (DTCS) with Quick Start Kit. The species of the isolates was identified with the use of BLAST GenBank (http://www.ncbi.nlm.nih.gov/blast/) and Ribosomal Database Project (http://rdp.cme.msu.edu/) software.

The following parameters were used to assess physiological/biochemical properties: enzymatic activity, optimal values of growth temperature and pH, capacity to produce auxins and to dissolve phosphorous compounds poorly accessible to plants. Protease, amylase and lipase activities were revealed following the standard procedures for assessing bacterial enzymatic activity [27, 28]. Cellulase activity was revealed following the described procedure [29]. Capacity for auxin production was tested with the use of the method described in [30] on the L–tryptophane–containing medium. Capacity to dissolve slightly soluble phosphorous compounds was tested on the Muromtsev medium [27] supplemented by 4.5 g of $Ca_3(PO4)_2$ and the phosphorite meal.

Studying the Establishment of Endophytic Bbacteria on Agricultural Crops in planta and Their Growth-promoting and Biocontrol Activity. To reveal the capacity of the studied strains of endophytic bacteria to colonize higher plants, seeds were inoculated with these bacteria and the plants were subsequently grown under the sterile conditions of gnotobiotic systems [31]. *Pseudomonas chlororaphis* RR228 strain from the collection of the Laboratory of Technical Microbiology (ARRIAM), which is already used in production of biopreparations, was used as a control strain for comparing the colonization activity of bacteria. The following crops were tested: wheat *Triticum aestivum* (Veda cultivar, selection by the Lukyanenko Krasnodar Research Institute of Agriculture), tomato *Solanum lycopersicum* (Persei cultivar, "Dom Semyan", St. Petersburg) and radish *Raphanus sativus* L. var. *radicula* (Duro cultivar, "Dom Semyan", St. Petersburg).

Wheat seeds were sterilized in 70% ethanol for 2 min, rinsed with sterile water and conditioned in 30% sodium hypochlorite solution twice for 30 min. Tomato and radish seeds were decontaminated with 70% ethanol for 1 min and conditioned in 30% sodium hypochlorite solution twice for 8 min. After sterilization the seeds were thoroughly washed in sterile water.

After that the seeds were inoculated with bacterial suspensions with a titre of 10^7 CFU/ml and grown in gnotobiotic systems following the procedures developed in ARRIAM [32]. To determine the number of introduced bacteria in the rhizoplane, rinsed roots were suspended in normal saline solution, with the abundance of microorganisms assessed by inoculation on solid R2A medium.

To study the growth-promoting activity, sterile seeds were soaked in the obtained bacterial suspensions for 30 min and then placed into sterile moist chambers on the surface of filter paper (in vitro experiment). In each variant 25 seeds were placed into one moist chamber and three replications were made. Seeds in the control were soaked in sterile normal saline solution. Seedlings were grown for 5 days at 28°C. Under conditions of the micro-vegetation experiment, tomato plants were grown in 3 l vessels with non-sterile peat soil ("Terravita", produced by Fart CJSC, Russia). The wet weight and the length of rootlets and seedling were measured and the difference between the experiment and the control was determined.

Spatial localization of bacteria on the roots of tomato plants was studied with the use of FISH and luminescent microscopy. For this, tomato rootlets were washed from sand and prepared for examination as described above. Hybridization was performed with

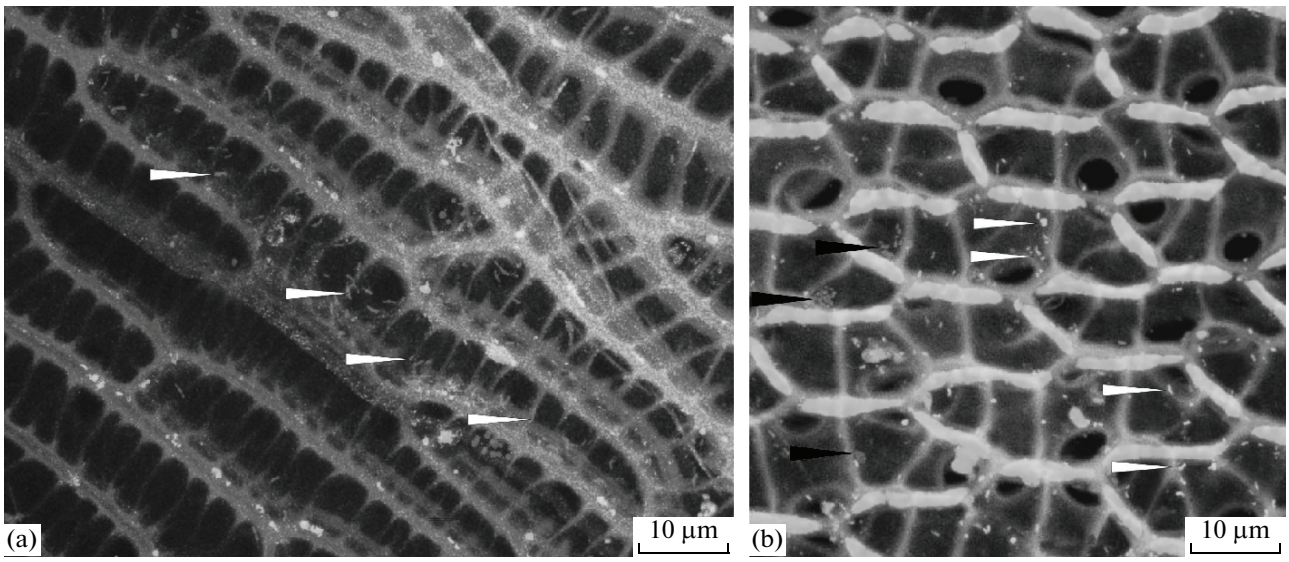

Fig. 1. Localization of bacteria in *Sphagnum* gametophytes. Fluorescent in situ hybridization of branch leaves: À, with universal bacterial probes EUB338, EUBII338, EUBIII338 (white arrows point to representatives of *Eubacteria*); B, with universal (EUB338) and group-specific (BET42a and GAM42a) bacterial probes (white arrows point to representatives of *Betaproteobacteria* and *Gammaproteobacteria*, black arrows, to other representatives *Eubacteria*). Confocal laser scanning microscopy images. Scale bar: 25 μm.

the use of the EUB338 universal bacterial probe [20]. The preparations were analyzed with the help of an epiluminescence microscope (Carl Zeiss, Germany).

In the experiment on the root rot biocontrol by *P. fluorescens* RF13H strain, the seeds of *Triticum aestivum* wheat (Podarok Donu cultivar) were sterilized superficially following the procedure described above and inoculated for 30 min with the suspension of *P. fluorescens* RF13H strain with a titre of 10^7 CFU/ml. The pathogenic background, that is, 1 ml of suspension of *Fusarium culmorum* spores with a titre of 10^5 CFU/ml, was introduced into sterile tubes filled with quartz sand moisturized up to 60% of the total water capacity. Inoculated seeds were planted into the sand 1 cm deep and the plants were grown in the phytotron for 7 days at 25°C. After that the plants were taken out and rinsed to remove sand; the maximum length of the aerial part and the roots was measured and the number of plants infected with root rot was registered.

The data were statistically treated with the help of DIANA (ARRIAM, St. Petersburg, Russia) and MS Excel 2007 (Microsoft Corporation, USA) software.

RESULTS AND DISCUSSION

Revealing Endophytic Bacteria in Moss Hyaline Cells

FISH followed by confocal microscopy made it possible to reveal colonization of the *Sphagnum* endosphere by microbial communities (Fig. 1).

Sphagnum gametophytes have a unique morphology. The leaves of all *Sphagnum* mosses consist of a single layer of alternating cells: narrow chlorophyllose cells performing photosynthesis and broad hollow hyaline cells, which have pores and perform structural and water-holding function. Three-dimensional reconstruction and subsequent image processing showed that endophytic bacteria were attached to the inner side of the walls of hyaline cells (Fig. 1).

The use of universal and group-specific probes allowed us to identify the representatives of the classes *Beta–* and *Gammaproteobacteria*, forming micro-colonies inside the hyaline cells. According to earlier data, β- and γ–proteobactria are a sub-dominant component of microbial communities associated with *Sphagnum* mosses [15]. Analysis of the libraries of 16S rRNA gene clones showed that these taxonomic groups made up, correspondingly, up to 7% and 12% of the total number of the revealed bacteria.

Isolation of Culturable Strains of Bacteria Associated with Sphagnum Mosses and Their Preliminary Characterization

In the course of our research, 283 strains of culturable forms of bacteria were isolated from moss plants collected in Austria and Russia. Their cultural/morphological characterization was studied. Most of the isolated bacteria were Gram-negative (more than 98%), rounded, oval or rod-shaped, and very small (<1.5 μm). On R2A medium these bacteria formed fast-growing, flat, creeping, transparent or semi-transparent colonies. Some of the colonies were

Table 2. Antagonistic activity of endophytic bacteria from *Sphagnum* mosses sampled in Voloyarvi Bog (3.10.2009) (an example). Percentage of strains with various degrees of antagonistic activity against the phytopathogens is given

Activity of phytopathogen suppression	Fungicidal activity				Bactericidal activity			
	F. culmorum	*F. sporotrichioides*	*F. graminearum*	*A. alternata*	*E. carotovora*	*P. syringae*	*P. fluorescens*	*C. michiganensis*
Active suppression, growth of the phytopathogen completely suppressed in the zone with $r > 10$ mm	45%	47%	42%	40%	18%	10%	16%	20%
Moderate suppression or inhibition, "pure" inhibition zones or growth inhibition in the zone with $r > 10$ mm	15%	15%	8%	10%	16%	14%	12%	22%
No antagonistic activity, phytopathogen develops around the well in the same way as in the control; its cultural/morphological features are unchanged	40%	38%	50%	50%	66%	76%	72%	58%

brightly coloured in shades of red, violet, pink, yellow and orange, while the others were colourless, beige or cloudy white. The total number of microorganisms grown on nutrient media varied in the range of 10^5–10^6 CFU/g of plant tissue. The growth of nitrogen-fixing and oligonitrophilous bacteria on nitrogen-free medium was rather abundant, but their morphotypes were few (never more than 5). They formed transparent, colourless, slimy middle-sized colonies, either creeping on the agar surface or rounded and prominent.

Screening of the isolated bacteria for antagonistic activity against a number of major phytopathogens revealed a high proportion of antagonistic strains (Table 2). More than 50% of the studied strains also had a pronounced fungicidal effect on *Fusarium* phytopathogenic fungi. More than 30% of the strains suppressed in various degrees the development of some common phytopathogenic bacteria. Several strains demonstrated a complex action, suppressing both fungal and bacterial phytopathogens, whereas several others had only a distinct bactericidal or a distinct fungicidal effect.

Molecular-genetic Identification and Physiological/Biochemical Characterization of the Most Promising Strains

Screening of the strains of endophytic bacteria from *Sphagnum* mosses possessing beneficial properties (Table 3) allowed us to select a number of promising ones. Molecular-genetic identification with the use of BLAST and RDP revealed a high diversity of these microorganisms. Dominating among them were species from the genera *Pseudomonas, Serratia, Burkholderia, Flavobacterium* and *Collimonas*. Noteworthy, representatives of the genus *Burkholderia* were also present among the studied strains. We found *B. bryophila* and *B. phenazinium* in association with *S. fallax* and *S. magellancum* from both Russia and Austria, which supports the hypothesis on highly specific association between *Burkholderia* bacteria and *Sphagnum* mosses [33]. Bacteria with a high antagonistic potential were identified as species of *Pseudomonas, Serratia* and *Flavobacterium*, the genera known for their biocontrol and plant growth promoting qualities and widely used in agricultural microbiology for development of broad-spectrum biopreparations [34–36].

The characterization of enzymatic activity of the studied bacterial strains showed that they possessed rather active hydrolytic enzymes. Strains actively releasing proteinases, amylases, lipases and cellulases were identified. The range of their optimum growth temperatures was 18–28°C, the growth being completely suppressed at +37°C. Some of the strained could grow psychrophilically at +4°C, which is probably explained by the temperature regimes of peatland in cold regions. The optimum pH lay in the range of 5.5–7.0, with the growth of all the strains being considerably suppressed at pH = 8.0. The capacity to accumulate auxins on L-tryptophane-containing medium was noted in six studied strains in vitro, indicating the potential of their use for growth promotion. Eight promising strains could dissolve slightly soluble phosphorus compounds such as calcium orthophosphate and phosphorite meal. These strains (mostly *Pseudomonas* representatives) will be used for the development of microbiological fertilizers with rock phosphate-mobilizing capacity.

Studying the Capacity of Sphagnum-associated Endophytic Bacteria to Colonize Agricultural Crops and Promote their Growth and Development

The capacity of bacterial strains isolated from *Sphagnum* mosses to colonize the rhizosphere of higher plants used as agricultural crops was always in the

Table 3. Taxonomic position and economic and physiological characteristics of the most promising strains of endophytic bacteria associated with *Sphagnum* mosses

Strain	Fungicidal activity, total	Bactericidal activity, total	Enzymatic activity	Ability to dissolve phosphates, $Ca_3(PO_4)_2$/ phosphorite meal	Growth on nitrogen-free media	Production of auxins, on L-tryptophane-containing medium	Temperature optimum	Growth at + 37°C	Growth at + 4°C	pH optimum
Pseudomonas brenneri RM14B	+++	++	P(+), L(+)	+/+	–	+	28	–	+	6.0
Pseudomonas poae RF11D	++	+++	P(++)	+/+	–	–	28	–	–	6.0
Pseudomonas fluorescens RF13H	+++	++	A(+++), L(++)	–/–	–	+	28	–	+	6.0
Serratia plymuthica AF24A	++	++	P(++), A(+), L(++)	–/–	–	+	28	–	+	7.0
Pseudomonas poae RM13C	+++	+	P(+), A(+)	+/+	–	–	18	–	–	5.0
Pseudomonas. sp. RM13D	+++	+	P(++)	+/+	–	–	28	–	–	6.0
Pseudomonas fluorescens RF14J	++	+	P(++), L(+)	+/+	–	–	28	–	+	6.0
Pseudomonas poae RF12C	+++	++	A(++)	+/+	–	–	28	–	+	6.0
Flavobacterium saccharophilum RM14A	–	+++	P(++), A(+++), L(++)	–/–	–	–	18	–	+	7.0
F. saccharophilum RF14A	–	+++	P(+), A(++), L(++), C(++)	–/–	–	–	28	–	–	7.0
Flavobacterium sp. NFX	–	–	P(+), A(+)	±/+	+	–	28	–	–	6.0
Burkholderia bryophila AM11B	++	–	A(+), L(++)	–/–	+	–	18	–	–	5.0
Burkholderia bryophila AM32A	–	–	A(+)	–/–	+	–	28	–	–	4.5
Burkholderia phenazinum RM13B	++	–	A(+)	–/–	–	–	28	–	–	6.0
Collimonas sp. RF12H	++	±	A(+++), L(++)	–/–	–	–	28	–	–	6.0
Collimonas sp. RF13F	++	–	P(+), L(+)	–/–	+	–	18	–	–	6.0
Collimonas sp. RM14C	+	++	P(+), L(+)	–/–	+	–	18	–	–	6.0
Collimonas sp. RF13G	++	–	–	–/–	–	–	28	–	–	6.0
Stenotrophomonas rhizophila RF13C	–	–	P(++), A(++)	–/–	–	–	28	–	±	6.0
Pedobacter cryoconitis RM11D	+	++	P(++), A(++), C(+++)	–/–	–	–	18	–	+	7.0
Pedobacter sp. RF14C	+	+	P(++), À(++), L(++)	–/–	–	–	28	–	+	7.0
Pedobacter kribbensis RF14G	+	–	P(+), A(+)	–/–	+	–	18	–	–	6.0
Pseudomonas sp. RF14D	++	–	P(+), A(+)	–/–	–	+	28	–	–	6.0
Pseudomonas poae RF14H	++	+	–	+/+	–	–	28	–	+	6.0
Pedobacter sp. RM12B	+	+	C(+++)	–/–	–	+	28	–	+	6.0
Chryseobacterium soli RM12F	+	–	P(++), A(+++), L(+)	–/–	–	+	28	–	–	6.0

Note: Degree of phytopathogen suppression: "–", no suppression; "+", up to 5 mm; "++", 5–15 mm; "+++", more than 15 mm. Enzymatic activity: "P", protease; "A", amylase; "L", lipase; "C", cellulase

Table 4. Establishment of type strains of endophytic bacteria from Sphagnum mosses on the roots of wheat and tomato

Strain	Establishment in the rhizosphere and on the roots of wheat			Establishment on tomato roots
	Abundance of bacteria, 10^5 CFU/plant		Proportion, rhizosphere/root, %	Abundance of bacteria, 10^5 CFU/plant
	Rhizosphere	Root		
Pseudomonas chlororaphis RR228 (control strain from the VNIICKhM collection)	67.0 ± 5.0	8.2 ± 0.6	12.2	19.5 ± 4.0
Serratia plymuthica AM24A	72.0 ± 6.0	10.2 ± 1.1	14.4	58.9 ± 5.3
Pseudomonas poae RF11D	56.0 ± 6.0	4.6 ± 0.5	8.2	30.2 ± 4.6
Flavobacterium saccharophilum RM14A	50.2 ± 2.0	8.0 ± 2.0	6.1	10.6 ± 4.6
Pseudomonas fluorescens RF13H	102.0 ± 9.0	8.6 ± 0.6	8.4	4.3 ± 2.0
Stenotrophomonas rhizophila RF13C	109.3 ± 11.0	15.5 ± 11.3	14.2	123.3 ± 10.1

focus of the our study. The colonization activity is crucial for the effectiveness of the strains with an increased antagonistic potential under conditions of the "higher plant–bacterium" system.

The colonization potential of the five tested bacterial strains isolated from *Sphagnum* mosses was rather high (Table 4). These strains successfully colonized both the rhizosphere and the roots of wheat as well as the surface of tomato roots, being at least as effective, and sometimes more effective, in this respect as the control strain *P. chlororaphis* RR228. Maximum values of establishment on wheat and tomato roots were registered for *Serratia plymuthica* AM24A and *Stenotrophomonas rhizophila* RF13C. Relative values of the abundance of these bacteria on the roots as compared with the rhizosphere were also maximum, making up 14%. The abundance of the other strains was also high, being comparable with the colonizing activity of the control strain. The average abundance of all the strains on tomato roots was several times higher than on wheat roots.

As shown by epiluminescent microscopy, strains of *Serratia plymuthica* AM24A, *Flavobacterium saccharophilum* RM14A, *Pseudomonas brenneri* RM14B, *Pseudomons poae* RF11D, *Collimonas* sp. RM14C actively colonized the surface of tomato roots (Fig. 2) as micro-colonies or biofilms arranged along the root axis. A characteristic feature of these micro-colonies was that they formed at the interfaces of the rhizodermal cells. At the same time, the bacterial colonies were spread along the longitudinal axis of the root, lying rather deep at the junctions of the plant cells. Single bacteria were also found on the surface of root hairs, but their numbers there were considerably lower than directly on the surface of the rhizoderm.

After screening of the bacterial isolates with growth-promoting activity, ten strains were selected. Inoculation of seeds with these bacterial cultures promoted rootlet growth in tomato seedlings and enhanced their total raw biomass in a laboratory experiment (Table 5). Under conditions of a micro-vegetation experiment with non-sterile soil, seed inoculation with these strains was also shown to have a positive effect on tomatoes. Statistically significant stimulation of plant development was shown for most of the ten selected strains, the increase in the plant biomass being 10–80%.

In the experiment on biocontrol of the wheat root rot caused by *Fusarium culmorum*, inoculation of wheat seeds with *P. fluorescens* RF13H statistically significantly decreased the prevalence of the disease, from 90 to 40% (Table 6). It should be noted that in the variant where the seeds were not treated with *F. ñulmorum*, about 50% of the seeds had some internal infection (presumably caused by *Alternaria* sp.), which was not removed after superficial sterilization. Inoculation by the studied strain completely suppressed the development of the internal infection in this variant.

To sum up, our results indicate that most of the isolated bacterial strains have a powerful antagonistic effect on a number of phytopathogens. This effect may underlie the unique ability of *Sphagnum* mosses to withstand bacterial and fungal diseases. The activity of these bacteria and the accumulation of their metabolites in moss tissues may ensure exceptional bactericidal and fungicidal properties of these mosses.

We demonstrated for the first time that bacteria isolated from *Sphagnum* mosses may actively colonize agricultural crops, promoting their growth and development. Colonization of plant roots was visualized with the use of gnotobiotic systems, FISH and epiluminescent microscopy.

Moreover, a number of promising bacterial strains with a complex of beneficial properties was selected for further study. These strains will be used for the development of pilot samples of biopreparations to be tested in various micro-vegetation and field experiments on crops with the aim of increasing their yield

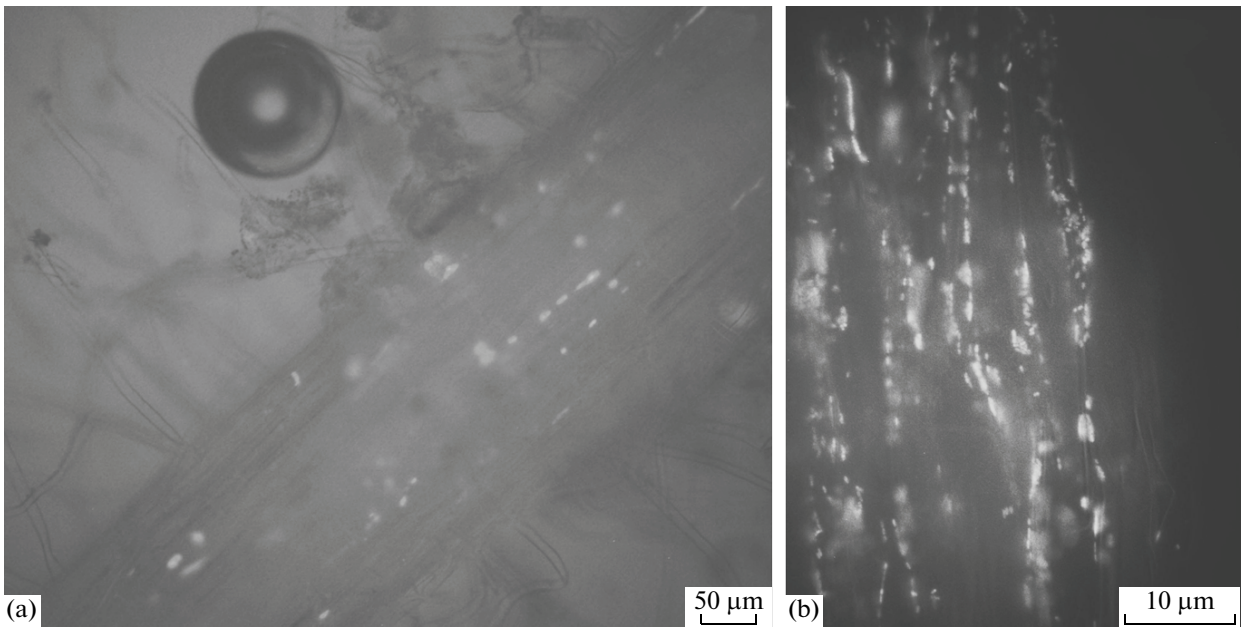

Fig. 2. Localization of the studied bacterial strains on tomato root surface with the use of fluorescent in situ hybridization and epiluminescent microscopy. A, tomato root with micro-colonies of *Pseudomonas fluorescens* RF13H arranged along its longitudinal axis. Scale bar: 50 μm. B, micro-colonies and single cells of *Pseudomonas fluorescens* RF13H on the surface of tomato roots. Scale bar: 10 μm. Hybridization made with the help of universal oligonucleotide probe EUB338 with Cy3 fluorochrome.

and protection against bacterial and fungal diseases. Seven strains were deposited with the Russian Collection of Agricultural Microorganisms (RCAM) of the ARRIAM (http://arriam.spb.ru/) (RCAM strain numbers 01009–01015).

ACKNOWLEDGMENTS

We are grateful to Dr.Vladimir B. Petrov, Dr. Vera I. Safronova, Dr. Evgeniy E. Andronov, Dr. Alexander G. Pinaev and Dr. Victor E. Tsyganov (ARRIAM), Tatyana Yu. Gagkaeva and Alexander M. Lazarev

Table 5. Stimulating effect of the studied bacterial strains on agricultural plants *in vitro* and under conditions of micro-vegetation experiment

Strain	Stimulation of radish seedlings *in vitro*		Stimulation in micro-vegetation experiment	
	Average rootlet length, mm	Total raw weight of the seedling, mg	Average raw weight of 12–days–old tomato plants, mg	Average dry weight of tomato plants (12 days of growth), mg
Control, untreated	13.1 ± 6.1	50.5 ± 14.6	194.1 ± 23.0	20.6 ± 1.7
Pseudomonas chlororaphis RR228 (control strain from the VNIICKhM collection)	Not studied	Not studied	310.7 ± 23.0 (+60%)	24.6 ± 1.7 (+19.4%)
Pseudomonas brenneri RM14B	13.8 ± 8.1	55.0 ± 19.3	374.2 ± 23.0 (+92%)	31.1 ± 1.7 (+52%)
Pseudomonas poae RF11D	18.5 ± 6.0	59.3 ± 15.0	275.9 ± 23.0 (+42%)	23.3 ± 1.7 (+13%)
Pseudomonas fluorescens RF13H	21.1 ± 5.7	64.7 ± 13.7	316.6 ± 23.0 (+63%)	27.8 ± 1.7 (+35%)
Pseudomonas fluorescens RF14J	24.8 ± 6.1	59.6 ± 13.7	321.2 ± 23.0 (+65%)	29.2 ± 1.7 (+41%)
Serratia plymuthica AM24A	30.0 ± 8.1	69.4 ± 17.3	229.7 ± 23.0 (+18%)	20.7 ± 1.7 (+0.5%)
Flavobacterium saccharophilum RM14A	16.8 ± 5.7	58.1 ± 13.7	252.1 ± 23.0 (+29.9%)	21.2 ± 1.7 (+2.9%)
Stenotrophomonas rhizophila RF13C	31.6 ± 9.4	89.6 ± 22.3	305.9 ± 23.0 (+57%)	26.2 ± 1.7 (+27%)
Collimonas sp. RF14Ñ	22.5 ± 6.1	68.4 ± 14.6	243.3 ± 23.0 (+25%)	23.5 ± 1.7 (+14%)

Table 6. A decrease in the prevalence of wheat root rot induced by *Fusarium culmorum* after inoculation with *Pseudomonas fluorescens* RF13H

Variant	Average shoot length, mm	Average root length, mm	Germinating power, %	Number of affected plants, %
Control	14.2 ± 2.8	6.9 ± 1.6	98	50, internal seed infection
Strain RF13H	14.4 ± 2.8	7.0 ± 1.6	93	0
Fusarium culmorum	6.7 ± 2.8	5.9 ± 1.6	91	90
Fusarium culmorum + strain RF13H	10.1 ± 2.8	6.6 ± 1.6	98	40

(VIZR) and the staff of Bisolbi-Inter LLC. The research was supported by the Russian Federation for Basic Research (grant no. 09–04–91007–ANF_a) and the State Contract of the Ministry for Education and Science no. 16.Ì04.11.0013 of 29.04.2011. It was carried out using the facilities of the Common Use Centre "Genome Technologies and Cell Biology" of the Agriculture Department of the Russian Academy of Agricultural Sciences (State Contract no. 16.552.11.7047).

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