

# The bryophyte genus *Sphagnum* is a reservoir for powerful and extraordinary antagonists and potentially facultative human pathogens

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## Keywords

bryophytes; bacterial antagonists; *Sphagnum*; *Verticillium dahliae*; *Rhizoctonia solani*.

## Abstract

*Sphagnum* plants grow in natural, species-poor carpets at low pH but without any known substantial fungal disease. To investigate this phenomenon, we analysed bacterial populations associated with two *Sphagnum* species with different ecological behaviour, namely *S. magellanicum* and *S. fallax*, from three sites in Germany and three in Norway, with a special focus on the functional group of antagonists. The screening of 493 bacterial isolates for antagonistic activity against fungal pathogens resulted in 237 (48%) active isolates. We found a higher proportion of antagonists for *S. magellanicum* (24%) than we did for *S. fallax* (19%) in general. The majority of the antagonists belonged to the genera *Serratia* (15%), *Burkholderia* (13.5%), *Staphylococcus* (13.5%), and *Pseudomonas* (10%). In contrast to the high moss specificity found for antagonistic bacteria, *Burkholderia* as well as *Serratia* isolates with highly similar molecular fingerprints as ascertained by BOX-PCR for both *Sphagnum* species were found. Interestingly, a high proportion of antagonists, for example *Staphylococcus*, *Hafnia*, *Yersinia*, and *Pantoea*, were identified as strains that are known as facultative pathogens of humans. *Sphagnum* plants represent an ecological niche not only for diverse and extraordinary microbial populations with a high potential for biological control of plant pathogens but also for opportunistic human pathogens.

## Introduction

The bryophyte genus *Sphagnum* is distributed worldwide and is the dominant component of peat-bog vegetation (Daniels & Eddy, 1985). Peat bogs belong to the oldest vegetation form, with maintenance for more than 1000 years. *Sphagnum* plants form an extreme habitat for microorganisms, characterized by high acidity (pH 3.5–5.0), low temperature, and an extremely low concentration of mineral nutrients. Because of their antimicrobial activity, *Sphagnum* plants were used as a natural medicine in the old Indian and Maya cultures, and as wound dressing during the First and Second World Wars (Ando & Mastuo, 1984; Frahm, 2001). Interestingly, *Sphagnum* plants form species-poor carpets at low pH. Although they are colonized by diverse bryophilous ascomycetes, no substantial fungal diseases are known (Döbbeler, 1997). The antifungal activity of bacteria that are associated with natural habitats, especially with bryophytes, is still unclear. It is interesting to speculate whether

*Sphagnum* mosses harbour antifungal bacteria that take part in the pathogen defence, and to which genera such bacteria belong. For the rhizosphere this phenomenon is well known: antifungal microorganisms are enriched and form part of the defence of the plant against soil-borne fungal pathogens (Cook *et al.*, 1995; Berg *et al.*, 2002, 2006; Weller *et al.*, 2002). Preliminary studies concerning the diversity and antagonistic potential of bacteria associated with different bryophyte species have shown that, in contrast to other species, the moss *Sphagnum rubellum* Wils. is colonized by specific bacterial communities with an extremely high proportion of antagonistic isolates and with a dominance of *Burkholderia* strains (Opelt & Berg, 2004).

The study of plant-associated bacteria and their antagonistic potential is important not only for gaining an understanding of their ecological role and the interaction with plants, but also for future biotechnological applications, for example biological control of soil-borne plant pathogens or the isolation of bioactive compounds (Weller,

1988; Bloembergen & Lugtenberg, 2001). The soil-borne fungi *Verticillium dahliae* KLEB. (ascomycetes) and *Rhizoctonia solani* KÜHN (basidiomycetes) were selected as the model pathogens for our antagonism studies. Both fungi have an extremely broad host range and are dangerous pathogens, causing dramatic yield losses worldwide on many important crops (Tjamos *et al.*, 2000; Stevenson *et al.*, 2001). The universal phase-out of the broad-spectrum fumigant methyl bromide as a control measure for soil-borne pathogens is expected to have a major impact on the frequency of occurrence and level of damage caused by soil-borne pathogens (Martin, 2003). Therefore, alternative control methods for these pathogens are urgently needed for commercial crop production. An environmentally friendly alternative to protect roots against fungal pathogens is antagonist-mediated biological control (Weller, 1988; Emmert & Handelsman, 1999; Weller *et al.*, 2002). Antagonists are naturally occurring organisms with traits enabling them to interfere with pathogen growth, survival or infection (Chernin & Chet, 2002). Knowledge of the indigenous antagonistic potential of each plant species is therefore important for understanding the natural self-protection of plants, as is the detection of new antagonists that could form a basis for biocontrol.

The aim of this study was to analyse and characterize the functional group of antagonists associated with *Sphagnum* plants and to determine the extent to which the plant species and the geographical region influence this special group. We therefore analysed the bacterial communities associated with two bryophyte species with different ecological behaviour, namely *Sphagnum magellanicum* and *Sphagnum fallax*, from different geographical sites in Germany and Norway. Both bryophytes belong to the typical and very important vegetation in peat bogs (Daniels & Eddy, 1985). Bacterial isolates were obtained by cultivation on R2A agar and then screened for antagonism against the soil-borne pathogenic fungi *V. dahliae* and *R. solani*. Isolates with antagonistic activity were identified and characterized genotypically (1) to provide basic knowledge of *Sphagnum*-associated bacteria, and (2) to find new bacteria able to control soil-borne fungal pathogens.

## Materials and methods

### Sampling and isolation of the bacterial fraction

We chose two common European *Sphagnum* species (class *Sphagnopsida*, family *Sphagnaceae*) growing in light and wet mires but in different ecological situations (Table 1). Both species and their distinctive habitats are often found adjoining in the same mire or area. The ecological characteristics of the different habitats were characterized by various abiotic conditions, especially with regard to moisture, soil reaction, and nutrient content, with the help of the ecological

indicator values developed by Ellenberg *et al.* (1991) based on a four-square-metre species list around the collecting point. Adult gametophytes of the two bryophytes *Sphagnum magellanicum* BRID. (section *Sphagnum*) [SM], and *Sphagnum fallax* H. KLINGGR. (section *Cuspidata*) [SF] were sampled from three different natural habitats in the southwest of Norway and from three habitats in the northeast of Germany. The sampling locations are shown in Table 1. The samples of the two bryophytes from the Norwegian sites were collected in August 2004. The first sampling site was located near Etne (N1), the second near Fjaera (N2), and the third near Røldal (N3). In addition, gametophytes of both bryophytes were collected from the German sites in September 2004 from the natural reserves 'Schlichtes Moor' near Güstrow (G1), 'Dänschenburger Moor' near Sanitz (G2), and 'Ribnitzer Großes Moor' near Graal-Müritz (G3). The green living parts of the gametophytes were placed in sterile Petri dishes and transported to the laboratory, and then 5 g was transferred to a sterile stomacher bag. To extract the moss-associated bacteria from the gametophytes, 45 mL of sterile 0.85% NaCl was added, and samples were homogenized in a stomacher laboratory blender for 60 s at high speed (BagMixer; Interscience, St Nom, France). This suspension was used for cultivation-dependent investigation procedures.

### Isolation of moss-associated bacteria and determination of CFU

Microbial suspensions obtained by the procedure explained above were serially diluted with sterile 0.85% NaCl and plated onto R2A medium (Difco, Detroit, MI). Plates were incubated for 5 days at 20 °C, after which CFU were counted to calculate the mean number of colonies ( $\log_{10}$  CFU) based on fresh weight. Data were analysed for significance using U-test "Mann-Whitney" ( $P \leq 0.05$ ) and by two factor analysis of variance by Statistical Product and Service Solution (SPSS Inc., Chicago, ILL). Isolates obtained by plating were purified and stored at -70 °C in sterile broth containing 50% glycerol. Isolated bacteria were encoded by a combination of numbers and letters indicating: (1) location (G, Germany; N, Norway); (2) sampling site (1, Schlichtes Moor/Bjørkjenes; 2, Dänschenburger Moor/Sørdalen; 3, Ribnitzer Großes Moor/Seljestad); (3) microenvironment (SM, *Sphagnum magellanicum*; SF, *Sphagnum fallax*); (4) consecutive number of the isolate per plant.

### Screening of antagonistic bacteria

Bacterial isolates were screened for their activity towards *V. dahliae* KLEB. and *R. solani* KÜHN by a dual-culture *in vitro* assay on Waksman agar (WA) containing 5 g of proteose-peptone (Merck, Darmstadt, Germany), 10 g of glucose (Merck), 3 g of meat extract (Chemex, Munich, Germany), 5 g of NaCl (Merck), 20 g of agar (Difco), and distilled water

**Table 1.** Sampling locations and some ecological characteristics of the various habitats

Abbreviation of collecting point*	<i>Sphagnum</i> species	State, province	District, locality, coordinates	Name/habitat	Moisture value <sup>†</sup>	Reaction value <sup>†</sup>	Nutrient value <sup>†</sup>
N1SM	<i>Sphagnum magellanicum</i>	Norway, Hordaland province	Etne district, 8 km east of Etne 59°39'43" N, 5°48'5" E	Bjørkenes peat bog	7.4	1.3	1.4
N2SM			Etne district, 5 km northeast of Fjæra 59°43'48" N, 6°28'36" E	Sørdalen peat bog	8.0	1.4	1.2
N3SM				Odda district, 10 km northwest Røldal 59°53'24" N, 6°38'5" E	Seljestad peat bog	7.8	1.7
G1SM		Germany, Mecklenburg-Western Pomerania	County of Güstrow, Niegleve 53°48'30" N, 12°21'25" E	Schlichtes Moor	7.7	1.6	1.0
G2SM			County of Nordvorpommern, Sanitz 54°07'30" N 12°25'45" E	Dänschenburger Moor	8.0	1.4	1.3
G3SM			County of Bad Doberan, Graal Müritz 54°16'15" N 12°17'30" E	Großes Ribnitzer Moor	8.1	1.7	1.6
N1SF	<i>Sphagnum fallax</i>	Norway, Hordaland province	Etne district, 8 km east of Etne 59°39'43" N, 5°48'5" E	Bjørkenes peat bog	7.4	2.8	2.8
N2SF			Etne district, 5 km northeast of Fjæra 59°43'48" N, 6°28'36" E	Sørdalen peat bog	7.5	2.4	2.2
N3SF				Odda district, 10 km northwest Røldal 59°53'24" N, 6°38'52" E	Seljestad peat bog	7.9	3.0
G1SF		Germany, Mecklenburg-Western Pomerania	County of Güstrow, Niegleve 53°48'30" N, 12°21'25" E	Schlichtes Moor	7.9	2.6	1.8
G2SF			County of Nordvorpommern, Sanitz 54°07'30" N, 12°25'45" E	Dänschenburger Moor	7.8	2.3	2.0
G3SF			County of Bad Doberan, Graal Müritz 54°16'15" N, 12°17'30" E	Großes Ribnitzer Moor	8.0	3.0	2.0

\*Letters represent the locations and microhabitats. G, Germany; N, Norway; SF, *Sphagnum fallax*; SM, *Sphagnum magellanicum*; arabic numerals represent the sampling site (1–3).

<sup>†</sup>Average indicator values by Ellenberg *et al.* (1991) calculated on the basis of species lists of every 4 m<sup>2</sup> collecting point.

The figures indicates ecological gradients (moisture: 1, extremely dry; 9, wet; reaction: 1, extremely acidic; 9, calcareous; nutrient: 1, extremely nutrient-poor; 9, extremely nutrient-rich).

(to 1 L) (pH 6.8). Zones of inhibition were measured after 3, 5 and 7 days of incubation at 20 °C according to the method of Berg (1996). All strains were tested in three independent replicates with *V. dahliae* ELV25 (isolated from *Brassica napus* L. by K. Zeise and kept in the culture collection of the University of Rostock, Department of Microbiology) and with *R. solani*. (isolated from *Solanum tuberosum* and kept in the culture collection of the University of Rostock, Department of Microbiology). The two plant-pathogenic fungi were routinely grown on Sabouraud medium (Gibco, Paisley, Scotland) and stored at –70 °C in sterile broth containing 50% glycerol.

### Purification of DNA from antagonistic bacteria

Sterile glass beads (Sigma, 0.25–0.5 mm) and 300 µL of extraction buffer (containing 200 mM Tris, 200 mM NaCl, 25 mM EDTA, and 0.5% sodium dodecyl sulfate) were added to the bacterial material. The colonies were treated with a FastPrep<sup>TM</sup> instrument (Qbiogene BIO 101<sup>®</sup> systems, Karlsbad) for 20 s at level 4. One hundred and fifty microliters of 3 M sodium acetate was added, and the samples were shaken with a vortexer. The samples were

frozen for about 30 min, and then centrifuged for 5 min at 13 000 g. Finally, the DNA (supernatant) was purified by phenol–chloroform extraction and precipitation by isopropanol. The resulting pellet was dissolved in 50 µL of TE buffer and stored at –20 °C.

### Characterization of antagonists by amplified rDNA restriction analysis

Amplified rDNA restriction analysis (ARDRA) was used to group isolates at the genus level. The 16S rRNA genes of the bacterial antagonists were PCR-amplified with the universal eubacterial primers EubI (5'-GAG TTT GAT CCT GGC TCA G-3') and EubII (5'-AGA AAG GAG GTG ATC CAG CC-3'). The PCR conditions consisted of an initial denaturing cycle (95 °C, 5 min), nine amplification cycles (95 °C, 30 s; 52 °C, 30 s; 72 °C, 1 min 40 s), 19 amplification cycles (95 °C, 30 s; 52 °C, 30 s; 72 °C, 1 min 30+10 s), and a final elongation cycle (72 °C, 5 min). The restriction enzyme chosen was HhaI. The enzymatic reactions were digested for 3 h at 37 °C in 20-µL volumes containing 15 µL of the PCR product solution, 2 µL of commercially supplied

incubation buffer, 2.55  $\mu\text{L}$  of water, 0.2  $\mu\text{L}$  of  $100 \times$  BSA, and 0.25  $\mu\text{L}$  ( $20 \text{ U } \mu\text{L}^{-1}$ ) of HhaI. Restriction products were run on a 2% agarose gel (AppliChem, Darmstadt, Germany) in a  $1 \times$  Tris-borate-EDTA buffer for 5 h at  $100 \text{ V } \text{m}^{-1}$ . The resulting bands were made visible with ethidium bromide. Isolates showing the same band pattern were arranged to form a group. The reproducibility of the results was verified in at least two independent experiments.

### Identification of bacterial antagonists

Some representative isolates of each ARDRA group were chosen to identify the whole group by partially sequencing the 16S rRNA gene. For this, the 50- $\mu\text{L}$  reaction mixture contained at least 10  $\mu\text{L}$  of PCR SuperMIX Taq & Go (Qbiogene), 1  $\mu\text{L}$  of primer EubI-forward (5'-GAG TTT GAT CCT GGC TCA G -3') and 1  $\mu\text{L}$  of primer 907-reverse (5'-CCG TCA ATT C(AC)T TT(AG) AGT TT-3'), and 1  $\mu\text{L}$  of template. The PCR was performed as described above. The PCR products were purified using a GeneClean Spin Kit (Qbiogene, Bio 101, Carlsbad, CA) according to the manufacturer's protocol. DNA templates were sequenced using an Applied Biosystems 3130  $\times$  I Genetic Analyzer sequencer Data Collection v. 3.0, Sequencing Analysis v. 5. The 16S rRNA gene sequences were aligned with sequences of the NCBI sequence databases using the BLAST algorithm according to Altschul *et al.* (1997).

### BOX-PCR fingerprints

BOX-PCR (fingerprinting based on repetitive BOX elements, of unknown function, in the bacterial genome) was carried out as described by Rademaker and De Bruijn (1997). Using the BOXA1R primer (5'-CTA CGG CAA GGC GAC GCT GACG-3'), PCR amplification was performed with a Peltier Thermal Cycler PTC-200 (Biozym Diagnostic, Hessisch Oldendorf, Germany) with an initial denaturation step at  $95^\circ\text{C}$  for 6 min; 35 cycles of denaturation at  $94^\circ\text{C}$  for 1 min, annealing at  $53^\circ\text{C}$  for 1 min, extension at  $65^\circ\text{C}$  for 8 min; and a final extension at  $65^\circ\text{C}$  for 16 min. A 10- $\mu\text{L}$  aliquot of the amplified PCR product was separated by gel electrophoresis on 1.5% agarose gels in  $0.5 \times$  Tris-borate-EDTA buffer for 5 h, stained with ethidium bromide, and then photographed under UV transillumination. The reproducibility of the results was verified in at least two independent experiments.

### Computer-assisted cluster analysis

Computer-assisted evaluation of bacterial fingerprints generated by BOX-PCR was performed using the GELCOMPAR program (version 4.1; Applied Maths, Kortrijk, Belgium). The cluster analysis was carried out with a Pearson correlation matrix with the UPGMA (unweighted pair group method with arithmetic averages) algorithm.

### Statistics

All statistical analyses were tested for significance using the Mann – Whitney *U* test ( $P \leq 0.05$ ) with SPSS for Windows, release 9.0.1 (SPSS Inc., Chicago).

### Nucleotide sequence accession numbers

The nucleotide sequences determined in this study have been deposited in the EMBL Data Library under accession numbers AM042686–AM042690, AM042703–AM042712, AM048788–AM048801, AM182514–AM182532, AM182537–AM182562, AM183954–AM183966, AM231271–AM231275, and AM236593.

## Results

### Isolation of bacteria from moss gametophytes

CFU determined for moss samples were fairly similar for the both *Sphagnum* species [counts, expressed as  $\log_{10}$  CFU  $\text{g}^{-1}$  (fresh weight) of plant, were  $4.7 \pm 0.74$  to  $5.7 \pm 0.44$  for *S. fallax*, and  $4.8 \pm 0.53$  to  $5.7 \pm 0.25$  for *S. magellanicum*]. The bacterial abundances at the different geographical locations did not differ statistically significantly.

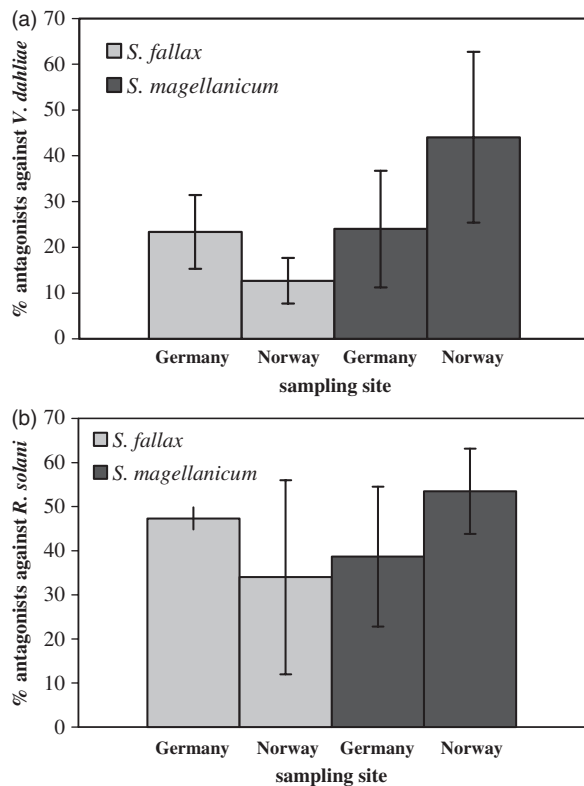
### Ecological characterization of the habitats

The habitats were characterized with the help of the ecological indicator values detailed in Ellenberg *et al.* (1991). Indicator values for moisture, soil reaction, and nutrient are expressed on a scale of 1–9 (moisture: 1, extremely dry; 9, wet; soil reaction: 1, extremely acidic; 9, calcareous; nutrient: 1, extremely nutrient-poor; 9, extremely nutrient-rich). The different habitats resemble each other in moisture content (*S. magellanicum*: 7.4–8.1 and *S. fallax*: 7.4–8.0), but differ in soil reaction and plant nutrient availability (Table 1). *Sphagnum magellanicum* (reaction value: 1.3–1.7; nutrient value 1.0–1.6) is typical for strong acidic, oligotrophic habitats, whereas *S. fallax* (reaction value: 2.3–3.0; nutrient value: 1.8–3.1) grows in weakly acidic, some mesotrophic situations influenced by minerotrophic groundwater.

### Screening for isolates antagonistic to *V. dahliae* and *R. solani*

A total of 493 bacterial isolates were screened for their ability to suppress *V. dahliae* and *R. solani* in an *in vitro* dual-culture assay. Initially, 237 (48%) antagonistic isolates were found: 95 (40%) of them were active against both pathogens, *V. dahliae* as well as *R. solani*. One hundred and eleven isolates (47%) showed antagonistic activity only against *R. solani*, and 31 isolates (13%) were active only against *V. dahliae*. The proportion of antagonistic bacteria was higher for *S. magellanicum* (24%) than for *S. fallax* (19%) and differs

statistically significantly between the two *Sphagnum* species at  $P \leq 0.1$ . The proportion of antagonistic isolates from *S. magellanicum* and *S. fallax* against *R. solani* or *V. dahliae* varied strongly, but did not differ significantly. For *S. magellanicum* the proportion of antagonistic isolates against *R. solani* was  $44 \pm 15.1\%$ , whereas  $32 \pm 18.8\%$  showed an activity against *V. dahliae*. For *S. fallax*  $40 \pm 21.1\%$  of the tested isolates showed an activity against *R. solani*, and only  $19 \pm 9.6\%$  were active against *V. dahliae*. Although similar numbers of isolates from each *Sphagnum* species and geographical site were tested, the proportions of isolates with antifungal activity were different. The proportion of isolates with antagonistic activity against *V. dahliae* (Fig. 1a) was highest for *S. magellanicum* from Norway ( $44 \pm 18.7\%$ ), followed by *S. magellanicum* from Germany ( $24 \pm 12.8\%$ ), *S. fallax* from Germany ( $23 \pm 8.1\%$ ), and *S. fallax* from Norway ( $13 \pm 5.0\%$ ). The proportion of isolates with antifungal activity against *R. solani* (Fig. 1b) was also highest for *S. magellanicum* from Norway ( $54 \pm 9.7\%$ ), followed by *S. fallax* from Germany ( $47 \pm 2.5\%$ ), *S. magellanicum* from Germany ( $39 \pm 15.9\%$ ), and *S. fallax* from Norway ( $34 \pm 21.5\%$ ). For both fungal pathogens, a higher proportion



**Fig. 1.** Proportion of *in vitro* (a) *Verticillium dahliae* and (b) *Rhizoctonia solani* antagonists determined in dual-culture assays of *Sphagnum magellanicum* and *Sphagnum fallax* in Germany and Norway. Error bars indicate SD.

of antagonists was found for *S. magellanicum* from Norway than from Germany. In contrast, for *S. fallax* the proportion of antagonists against *R. solani* or *V. dahliae* was highest for the German sites followed by the Norwegian ones, although these differences were not statistically significant.

### Characterization and identification of antagonistic bacterial isolates

In total, 155 bacterial isolates were characterized by 16S rRNA gene restriction fragment length polymorphism and could be assigned to 25 ARDRA types (A–Y). Twenty distinct ARDRA types were found for antagonists isolated from *S. fallax*, but only 16 distinct types for those originating from *S. magellanicum* (Table 2). Nine ARDRA types (H, M, R, S, T, U, V, W, Y) were found for antagonists isolated from *S. fallax*. In contrast, five ARDRA groups (J, N, P, Q, X) were specific for isolates from *S. magellanicum*. Eleven ARDRA groups (A–G, I, K, L, and O) were found for both *Sphagnum* species. The number of distinct ARDRA types was similar between both *Sphagnum* species and between geographical sites. For antagonistic bacterial isolates of *S. fallax* from Germany we found 12 distinct ARDRA types; for *S. fallax* from Norway, 15 types; for *S. magellanicum* from Germany, 13 types; and from Norway, 12 distinct ARDRA types. For identification, the 16S rRNA genes of 94 representative isolates comprising all ARDRA types were partially sequenced, and sequences were compared with entries available in public databases (Table 3). The 16S rRNA genes showed high homology to known sequences belonging to the *Betaproteobacteria* and *Gammaproteobacteria*, as well as to high and low G+C Gram-positive bacteria, and enteric bacteria. Thirty-six species belonging to 20 genera were found. The main ARDRA group A includes the majority of antagonistic isolates (23 of 155 = 15%), followed by ARDRA groups B and C (21 of 155 = 13.5%). The ARDRA type A was represented by the genus *Serratia*, dominantly found on *S. magellanicum*. All the representatives that were sequenced and aligned belonged to four *Serratia* species, namely *Se. plymuthica*, *Se. proteamaculans*, *Se. liquefaciens*, and *Se. grimesii*, or could be identified only at the genus level (*Serratia* spp.). The genus *Pseudomonas* (ARDRA type D) was dominantly found for antagonists from *S. fallax*. Isolates that were sequenced and aligned belong to four *Pseudomonas* species, namely *P. fluorescens*, *P. fragi*, *P. putida*, and *P. gingeri*. The genera *Staphylococcus* (ARDRA type B) and *Burkholderia* (ARDRA type C) were similarly found as antagonists from *S. fallax* and *S. magellanicum*. Isolates of the ARDRA group B could be identified as four *Staphylococcus* species, namely *St. pasteurii*, *St. epidermidis*, *St. caprae*, and *St. croceolyticus*, or could be identified only at the genus level (*Staphylococcus* spp.). Isolates belonging to *Serratia*, *Staphylococcus*,

**Table 2.** Number of isolates in and distribution of ARDRA groups

ARDRA group*	Genus†	No. of isolates	Origin‡			
			<i>Sphagnum fallax</i>		<i>Sphagnum magellanicum</i>	
			G	N	G	N
A	<i>Serratia</i>	23	2	2	2	17
B	<i>Staphylococcus</i>	21	9	2	7	3
C	<i>Burkholderia</i> I	21	7	3	4	7
D	<i>Pseudomonas</i> I	15	0	14	0	1
E	<i>Bacillus</i> I	13	6	3	3	1
F	<i>Chromobacterium</i> I	11	0	1	5	5
G	<i>Erwinia</i>	7	1	4	1	1
H	<i>Pseudomonas</i> II	5	5	0	0	0
I	<i>Hafnia</i>	5	1	0	4	0
J	<i>Burkholderia</i> II/ <i>Rothia</i>	4	0	0	3	1
K	<i>Bacillus</i> II	4	1	1	0	2
L	<i>Achromobacter</i>	4	2	0	2	0
M	<i>Pseudomonas</i> III	3	2	1	0	0
N	<i>Rahnella</i>	3	0	0	1	2
O	<i>Micrococcus</i> I	3	0	1	2	0
P	<i>Burkholderia</i> III	2	0	0	1	1
Q	<i>Chromobacterium</i> II	2	0	0	0	2
R	<i>Pantoea</i>	1	0	1	0	0
S	<i>Arthrobacter</i>	2	0	2	0	0
T	<i>Micrococcus</i> II	1	0	1	0	0
U	<i>Plantibacter</i>	1	0	1	0	0
V	<i>Fulvimonas</i>	1	1	0	0	0
W	<i>Dyella</i>	1	1	0	0	0
X	<i>Delftia</i>	1	0	0	1	0
Y	<i>Yersinia</i>	1	0	1	0	0
Σ ARDRA groups		25	12	15	13	12
Σ Isolates		155	38	38	36	43

\*Letters represent different restriction patterns of the 16S rRNA gene using HhaI.

†Genus was identified by partial 16S rRNA gene sequencing.

‡Origin: G, Germany; N, Norway.

*Burkholderia*, and *Pseudomonas* represented 61% of the selected antagonists and were found on both moss species. Furthermore, isolates of the genera *Bacillus*, *Chromobacterium*, *Erwinia*, *Hafnia*, *Achromobacter*, and *Micrococcus* were detected for both moss species. Antagonistic isolates of the genera *Pantoea*, *Arthrobacter*, *Plantibacter*, *Fulvimonas*, *Dyella*, and *Yersinia* were only found on *S. fallax*, whereas the genera *Rahnella*, *Rothia*, and *Delftia* were found only on *S. magellanicum* (Table 3).

### Characterization of antagonists belonging to the *Serratia* and *Burkholderia* groups by BOX-PCR

Antagonists of the genus *Serratia* (ARDRA group A) and *Burkholderia* (ARDRA group C) were characterized at the genotypic level using BOX-PCR. GELCOMP was used for

the comparison of BOX patterns. Antagonists assigned by 16S rRNA gene sequencing to the genus *Serratia* were isolated mainly from *S. magellanicum*. A total of 22 *Serratia* and 21 *Burkholderia* isolates were genotypically characterized by their BOX- fingerprints to detect moss-specific genotypes (Fig. 2). Analysis of BOX patterns for *Serratia* at a similarity of 80% resulted in nine distinct cluster or genotype groups (S1–9), although seven of them contained only one isolate (Fig. 2a). The cluster analysis of BOX fingerprints showed a high genotypic diversity and high plant specificity of the genus *Serratia* at the genotypic level. However, one group (S9) contained isolates from *S. magellanicum* (N3SM13) as well as from *S. fallax* (N2SF3). We therefore found the same genotype for isolates of the genus *Serratia* associated with different *Sphagnum* species. Genotype group S7 contained exclusive isolates associated with *S. magellanicum* from Norway ( $n = 13$ ).

The genotypic diversity of isolates could also be shown for the genus *Burkholderia*. A comparison of all BOX patterns generated from the *Burkholderia* antagonists of the ARDRA group C (Fig. 2b) resulted, at a similarity level of 80%, in 12 distinct clusters or genotypic groups (B1–12). Eight cluster groups (B3–5, and B8–10) contained only one isolate. The other four genotypic groups contained three (B1, B2, B7) or four (B6) isolates. Regarding the distribution of the isolates from different *Sphagnum* species, one group (B2) was formed by isolates from *S. fallax* only. In all other groups, isolates from both moss species were present (B1, B6, B7). Cluster group B6 could be divided into two subgroups at a similarity level of 93%, with one of the subgroups containing antagonists isolated from *S. magellanicum* (G3SM48) and from *S. fallax* (G3SF48) in Germany. This means that very highly similar BOX patterns were found for the isolates G3SM48 and G3SF48, which were isolated from different *Sphagnum* species in the same geographical region. Hence, we found the same genotypes for isolates of the genus *Burkholderia* on *S. magellanicum* and on *S. fallax*, and, not only did the isolates N2SM4, N2SM16 and G3SF45 show very highly similar BOX patterns, but the isolates G3SM38 and G3SF48 did too. In addition, for the isolates N2SM4, N2SM16, which were isolated from *S. magellanicum* in Norway, and G3SF45, which was isolated from *S. fallax* in Germany, we found the same genotype at different geographical sites. In conclusion, we found the same genotypes for isolates of the genus *Burkholderia* on different *Sphagnum* species and in different geographical regions.

**Table 3.** Taxonomic classification and characterization of bacterial isolates with antagonistic properties

No.	ARDRA group*	Strain†	Origin		Closest database match and accession number	SI‡	Taxonomic grouping	Activity§ against:	
			Location	Microenvironment				V. d.	R. s.
1	A	G1SF38	Schlichtes Moor	<i>Sphagnum fallax</i>	<i>Serratia plymuthica</i> DQ365586.1	99	<i>Enterobacteria</i>	–	+
2	A	G3SF33	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	ND			–	+
3	A	N1SF20	Bjorkjenes	<i>Sphagnum fallax</i>	ND			++	+
4	A	N2SF3	Sjordalen	<i>Sphagnum fallax</i>	<i>Serratia</i> sp. AY745744.1	98	<i>Enterobacteria</i>	+	–
5	A	G1SM11	Schlichtes Moor	<i>Sphagnum magellanicum</i>	<i>Serratia</i> sp. AY745744.1	99	<i>Enterobacteria</i>	–	++
6	A	G3SM26	Ribnitzer Großes Moor	<i>Sphagnum magellanicum</i>	<i>Serratia proteamaculans</i> AJ233435.1	99	<i>Enterobacteria</i>	–	+
7	A	N1SM4	Bjorkjenes	<i>Sphagnum magellanicum</i>	ND			+	++
8	A	N1SM5	Bjorkjenes	<i>Sphagnum magellanicum</i>	<i>Serratia proteamaculans</i> AY040208.1	98	<i>Enterobacteria</i>	+	++
9	A	N1SM7	Bjorkjenes	<i>Sphagnum magellanicum</i>	ND			+	++
10	A	N1SM9	Bjorkjenes	<i>Sphagnum magellanicum</i>	ND			–	++
11	A	N1SM20	Bjorkjenes	<i>Sphagnum magellanicum</i>	ND			++	++
12	A	N1SM25	Bjorkjenes	<i>Sphagnum magellanicum</i>	<i>Serratia liquefaciens</i> AJ306725.1	98	<i>Enterobacteria</i>	+	–
13	A	N1SM26	Bjorkjenes	<i>Sphagnum magellanicum</i>	ND			+	++
14	A	N1SM29	Bjorkjenes	<i>Sphagnum magellanicum</i>	ND			–	+
15	A	N1SM32	Bjorkjenes	<i>Sphagnum magellanicum</i>	<i>Serratia liquefaciens</i> AJ306725.1	97	<i>Enterobacteria</i>	–	+
16	A	N1SM33	Bjorkjenes	<i>Sphagnum magellanicum</i>	ND			–	++
17	A	N1SM34	Bjorkjenes	<i>Sphagnum magellanicum</i>	<i>Serratia grimesii</i> AF286868.1	97	<i>Enterobacteria</i>	–	++
18	A	N1SM36	Bjorkjenes	<i>Sphagnum magellanicum</i>	<i>Serratia liquefaciens</i> AJ306725.1	98	<i>Enterobacteria</i>	–	++
19	A	N3SM13	Seljestad	<i>Sphagnum magellanicum</i>	<i>Serratia proteamaculans</i> AJ233435.1	99	<i>Enterobacteria</i>	++	++
20	A	N3SM22	Seljestad	<i>Sphagnum magellanicum</i>	ND			+	++
21	A	N3SM23	Seljestad	<i>Sphagnum magellanicum</i>	ND			+	++
22	A	N3SM28	Seljestad	<i>Sphagnum magellanicum</i>	ND			++	++
23	A	N3SM29	Seljestad	<i>Sphagnum magellanicum</i>	<i>Serratia grimesii</i> AF286868.1	98	<i>Enterobacteria</i>	++	++
24	B	G1SF18	Schlichtes Moor	<i>Sphagnum fallax</i>	<i>Staphylococcus pasteurii</i> AJ717376.1	99	<i>Firmicutes</i>	–	+
25	B	G1SF21	Schlichtes Moor	<i>Sphagnum fallax</i>	<i>Staphylococcus caprae</i> AB009935.1	99	<i>Firmicutes</i>	+	++
26	B	G1SF27	Schlichtes Moor	<i>Sphagnum fallax</i>	<i>Staphylococcus pasteurii</i> AY553127.1	98	<i>Firmicutes</i>	+	+
27	B	G1SF41	Schlichtes Moor	<i>Sphagnum fallax</i>	<i>Staphylococcus epidermidis</i> AJ717377.1	98	<i>Firmicutes</i>	–	+
28	B	G2SF6	Dänschenburger Moor	<i>Sphagnum fallax</i>	<i>Staphylococcus pasteurii</i> AJ717376.1	97	<i>Firmicutes</i>	+	+
29	B	G2SF44	Dänschenburger Moor	<i>Sphagnum fallax</i>	<i>Staphylococcus pasteurii</i> AJ717376.1	98	<i>Firmicutes</i>	+	–

Table 3. Continued.

No.	ARDRA group*	Strain†	Origin		Closest database match and accession number	SI‡	Taxonomic grouping	Activity§ against:	
			Location	Microenvironment				V. d.	R. s.
30	B	G3SF11	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	<i>Staphylococcus caprae</i> AB009935.1	99	Firmicutes	–	+
31	B	G3SF44	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	ND			++	+
32	B	G3SF50	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	<i>Staphylococcus pasteurii</i> AJ717376.1	98	Firmicutes	+	+
33	B	N1SF3	Bjørkjenes	<i>Sphagnum fallax</i>	ND			+	–
34	B	N1SF34	Bjørkjenes	<i>Sphagnum fallax</i>	<i>Staphylococcus croceolyticus</i> AY953148.1	98	Firmicutes	–	+
35	B	G1SM12	Schlichtes Moor	<i>Sphagnum magellanicum</i>	<i>Staphylococcus epidermidis</i> AY640306.1	99	Firmicutes	++	++
36	B	G1SM25	Schlichtes Moor	<i>Sphagnum magellanicum</i>	<i>Staphylococcus epidermidis</i> AY640306.1	98	Firmicutes	+	++
37	B	G2SM10	Dänschenburger Moor	<i>Sphagnum magellanicum</i>	<i>Staphylococcus pasteurii</i> AJ717376.1	97	Firmicutes	++	++
38	B	G2SM11	Dänschenburger Moor	<i>Sphagnum magellanicum</i>	<i>Staphylococcus</i> sp. DQ170437.1	98	Firmicutes	–	+
39	B	G2SM26	Dänschenburger Moor	<i>Sphagnum magellanicum</i>	ND			++	++
40	B	G2SM27	Dänschenburger Moor	<i>Sphagnum magellanicum</i>	ND			++	++
41	B	G2SM43	Dänschenburger Moor	<i>Sphagnum magellanicum</i>	<i>Staphylococcus</i> sp. AB192377.1	98	Firmicutes	+	+
42	B	N2SM5	Sørdalen	<i>Sphagnum magellanicum</i>	ND			+	–
43	B	N3SM21	Seljestad	<i>Sphagnum magellanicum</i>	<i>Staphylococcus croceolyticus</i> AY953148.1	98	Firmicutes	+	++
44	B	N3SM26	Seljestad	<i>Sphagnum magellanicum</i>	<i>Staphylococcus</i> sp. DQ207366.1	98	Firmicutes	+	++
45	C	G1SF19	Schlichtes Moor	<i>Sphagnum fallax</i>	ND			–	++
46	C	G1SF32	Schlichtes Moor	<i>Sphagnum fallax</i>	ND			+	+
47	C	G3SF2	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	<i>Burkholderia phenazinium</i> AM086228.1	98	Beta-Proteobacteria	+	++
48	C	G3SF5	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	ND			+	++
49	C	G3SF30	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	ND			–	+
50	C	G3SF45	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	<i>Burkholderia</i> sp. AJ971347.1	99	Beta-Proteobacteria	–	+
51	C	G3SF48	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	<i>Burkholderia phenazinium</i> AY154375.1	98	Beta-Proteobacteria	–	+
52	C	N1SF5	Bjørkjenes	<i>Sphagnum fallax</i>	ND			+	–
53	C	N1SF40	Bjørkjenes	<i>Sphagnum fallax</i>	<i>Burkholderia</i> sp. AJ971350.1	97	Beta-Proteobacteria	++	–
54	C	N3SF47	Seljestad	<i>Sphagnum fallax</i>	<i>Burkholderia</i> sp. AJ971350.1	97	Beta-Proteobacteria	++	+
55	C	G1SM39	Schlichtes Moor	<i>Sphagnum magellanicum</i>	ND			++	–
56	C	G2SM45	Dänschenburger Moor	<i>Sphagnum magellanicum</i>	<i>Burkholderia</i> sp. AJ971350.1	97	Beta-Proteobacteria	–	+
57	C	G3SM43	Ribnitzer Großes Moor	<i>Sphagnum magellanicum</i>	<i>Burkholderia phytofirmans</i> AM0086238.1	98	Beta-Proteobacteria	+	+
58	C	G3SM48	Ribnitzer Großes Moor	<i>Sphagnum magellanicum</i>	ND			++	–
59	C	N1SM16	Bjørkjenes	<i>Sphagnum magellanicum</i>	<i>Burkholderia phenazinium</i> U96936.1	99	Beta-Proteobacteria	++	+++
60	C	N1SM17	Bjørkjenes	<i>Sphagnum magellanicum</i>	<i>Burkholderia phytofirmans</i> AM0086238.1	99	Beta-Proteobacteria	+	–
61	C	N2SM4	Sørdalen	<i>Sphagnum magellanicum</i>	<i>Burkholderia</i> sp. AJ971350.1	99	Beta-Proteobacteria	+	++



Table 3. Continued.

No.	ARDRA group*	Strain <sup>†</sup>	Origin		Closest database match and accession number	SI <sup>‡</sup>	Taxonomic grouping	Activity <sup>§</sup> against:	
			Location	Microenvironment				V. d.	R. s.
62	C	N2SM16	Sørdalen	<i>Sphagnum magellanicum</i>	ND			–	++
63	C	N3SM4	Seljestad	<i>Sphagnum magellanicum</i>	<i>Burkholderia terricola</i> AM086244.1	98	Beta-Proteobacteria	+	++
64	C	N3SM24	Seljestad	<i>Sphagnum magellanicum</i>	ND			+	++
65	C	N3SM30	Seljestad	<i>Sphagnum magellanicum</i>	<i>Burkholderia</i> sp. AJ971350.1	98	Beta-Proteobacteria	–	++
66	D	N1SF4	Bjørkjenes	<i>Sphagnum fallax</i>	<i>Pseudomonas fluorescens</i> AF094730.1	98	Gamma-Proteobacteria	–	+
67	D	N1SF9	Bjørkjenes	<i>Sphagnum fallax</i>	<i>Pseudomonas gingeri</i> AF320991	99	Gamma-Proteobacteria	–	+
68	D	N1SF10	Bjørkjenes	<i>Sphagnum fallax</i>	ND			–	+
69	D	N1SF11	Bjørkjenes	<i>Sphagnum fallax</i>	ND			–	+
70	D	N1SF12	Bjørkjenes	<i>Sphagnum fallax</i>	ND			–	+
71	D	N1SF14	Bjørkjenes	<i>Sphagnum fallax</i>	ND			–	+
72	D	N1SF25	Bjørkjenes	<i>Sphagnum fallax</i>	<i>Pseudomonas fluorescens</i> AF094730.1	99	Gamma-Proteobacteria	–	+
73	D	N1SF32	Bjørkjenes	<i>Sphagnum fallax</i>	<i>Pseudomonas fluorescens</i> AF094730.1	98	Gamma-Proteobacteria	–	+
74	D	N1SF33	Bjørkjenes	<i>Sphagnum fallax</i>	nd			–	+
75	D	N1SF39	Bjørkjenes	<i>Sphagnum fallax</i>	<i>Pseudomonas fluorescens</i> AF094730.1	98	Gamma-Proteobacteria	–	+
76	D	N1SF42	Bjørkjenes	<i>Sphagnum fallax</i>	<i>Pseudomonas gingeri</i> AF320991	99	Gamma-Proteobacteria	–	+
77	D	N1SF44	Bjørkjenes	<i>Sphagnum fallax</i>	ND			–	++
78	D	N1SF50	Bjørkjenes	<i>Sphagnum fallax</i>	<i>Pseudomonas fluorescens</i> AM048788.1	98	Gamma-Proteobacteria	–	+
79	D	N3SF33	Seljestad	<i>Sphagnum fallax</i>	<i>Pseudomonas putida</i> AY958233.1	98	Gamma-Proteobacteria	–	+
80	D	N1SM1	Bjørkjenes	<i>Sphagnum magellanicum</i>	<i>Pseudomonas fragi</i> AF094733.1	98	Gamma-Proteobacteria	+	+
81	E	G1SF6	Schlichtes Moor	<i>Sphagnum fallax</i>	<i>Bacillus pumilus</i> AY112667.1	98	Firmicutes	–	+
82	E	G1SF14	Schlichtes Moor	<i>Sphagnum fallax</i>	ND			–	++
83	E	G1SF17	Schlichtes Moor	<i>Sphagnum fallax</i>	<i>Bacillus</i> sp. DQ180948.1	97	Firmicutes	–	+
84	E	G1SF39	Schlichtes Moor	<i>Sphagnum fallax</i>	<i>Bacillus</i> sp. AF290561.1	99	Firmicutes	–	+
85	E	G2SF17	Dänschenburger Moor	<i>Sphagnum fallax</i>	<i>Bacillus pumilus</i> DQ188940.1	97	Firmicutes	+	+
86	E	G2SF32	Dänschenburger Moor	<i>Sphagnum fallax</i>	ND			+	+
87	E	N1SF2	Bjørkjenes	<i>Sphagnum fallax</i>	ND			++	+
88	E	N1SF21	Bjørkjenes	<i>Sphagnum fallax</i>	ND			–	+
89	E	N1SF23	Bjørkjenes	<i>Sphagnum fallax</i>	<i>Bacillus pumilus</i> AY112667.1	99	Firmicutes	–	+
90	E	G1SM6	Schlichtes Moor	<i>Sphagnum magellanicum</i>	<i>Bacillus pumilus</i> DQ232736.1	98	Firmicutes	+	–
91	E	G1SM38	Schlichtes Moor	<i>Sphagnum magellanicum</i>	<i>Bacillus pumilus</i> DQ275671.1	99	Firmicutes	+	+
92	E	G2SM49	Dänschenburger Moor	<i>Sphagnum magellanicum</i>	ND			–	+
93	E	N2SM6	Sørdalen	<i>Sphagnum magellanicum</i>	<i>Bacillus pumilus</i> AY269870.1	99	Firmicutes	+	++
94	F	N1SF36	Bjørkjenes	<i>Sphagnum magellanicum</i>	<i>Chromobacterium</i> sp. AY117572.1	99	Beta-Proteobacteria	++	+++
95	F	G1SM15	Schlichtes Moor	<i>Sphagnum magellanicum</i>	<i>Chromobacterium</i> sp. AY117572.1	99	Beta-Proteobacteria	+	+
96	F	G1SM16	Schlichtes Moor	<i>Sphagnum magellanicum</i>	ND			+++	+
97	F	G1SM26	Schlichtes Moor	<i>Sphagnum magellanicum</i>	ND			–	+

Table 3. Continued.

No.	ARDRA group*	Strain†	Origin		Closest database match and accession number	SI‡	Taxonomic grouping	Activity§ against:	
			Location	Microenvironment				V. d.	R. s.
98	F	G1SM36	Schlichtes Moor	<i>Sphagnum magellanicum</i>	ND			+	+
99	F	G2SM50	Dänschenburger Moor	<i>Sphagnum magellanicum</i>	ND			++	++
100	F	N1SM2	Bjørkjenes	<i>Sphagnum magellanicum</i>	<i>Chromobacterium</i> sp. AY117572.1	99	Beta-Proteobacteria	+	-
101	F	N1SM3	Bjørkjenes	<i>Sphagnum magellanicum</i>	ND			+	-
102	F	N1SM12	Bjørkjenes	<i>Sphagnum magellanicum</i>	ND			+	-
103	F	N1SM23	Bjørkjenes	<i>Sphagnum magellanicum</i>	<i>Chromobacterium</i> sp. AM048793.1	99	Beta-Proteobacteria	+	-
104	F	N1SM28	Bjørkjenes	<i>Sphagnum magellanicum</i>	ND			+	++
105	G	G3F29	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	<i>Erwinia persicina</i> AJ937837.1	98	Enterobacteria	+	-
106	G	N1SF48	Bjørkjenes	<i>Sphagnum fallax</i>	<i>Erwinia rhapontici</i> U80206.1	97	Enterobacteria	++	-
107	G	N3SF16	Seljestad	<i>Sphagnum fallax</i>	<i>Erwinia persicina</i> U80205.1	97	Enterobacteria	+	-
108	G	N3SF18	Seljestad	<i>Sphagnum fallax</i>	ND			+	+
109	G	N3SF19	Seljestad	<i>Sphagnum fallax</i>	ND			-	+
110	G	G3SM27	Ribnitzer Großes Moor	<i>Sphagnum magellanicum</i>	ND			-	++
111	G	N3SM14	Seljestad	<i>Sphagnum magellanicum</i>	<i>Erwinia rhapontici</i> U80206.1	98	Enterobacteria	+	++
112	H	G2SF27	Dänschenburger Moor	<i>Sphagnum magellanicum</i>	<i>Pseudomonas</i> sp. AY014814.1	99	Gamma-Proteobacteria	++	++
113	H	G3SF9	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	<i>Pseudomonas salomonii</i> AY091528.1	98	Gamma-Proteobacteria	-	++
114	H	G3SF16	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	ND			-	++
115	H	G3SF17	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	<i>Pseudomonas</i> sp. AY014814.1	99	Gamma-Proteobacteria	+	+
116	H	G3SF23	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	ND			+	+
117	I	G1SF12	Schlichtes Moor	<i>Sphagnum fallax</i>	<i>Hafnia alvei</i> AY253922.1	98	Enterobacteria	-	++
118	I	G1SM33	Schlichtes Moor	<i>Sphagnum magellanicum</i>	ND			++	+
119	I	G2SM44	Dänschenburger Moor	<i>Sphagnum magellanicum</i>	ND			-	+
120	I	G3SM15	Ribnitzer Großes Moor	<i>Sphagnum magellanicum</i>	<i>Hafnia alvei</i> AY253922.1	98	Enterobacteria	++	-
121	I	G3SM25	Ribnitzer Großes Moor	<i>Sphagnum magellanicum</i>	<i>Hafnia alvei</i> AY253922.1	99	Enterobacteria	-	++
122	J	G1SM18	Schlichtes Moor	<i>Sphagnum magellanicum</i>	ND			+	+
123	J	G1SM47	Schlichtes Moor	<i>Sphagnum magellanicum</i>	<i>Burkholderia phenazinium</i> AJ575090.1	98	Beta-Proteobacteria	-	+
124	J	G2SM46	Dänschenburger Moor	<i>Sphagnum magellanicum</i>	<i>Burkholderia multivorans</i> AY486372.1	98	Beta-Proteobacteria	++	+
125	J	N2SM10	Sørdalen	<i>Sphagnum magellanicum</i>	<i>Rothia amarae</i> AY043359.1	99	Actinobacteria	-	+
126	K	G3SF4	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	ND			-	+
127	K	N1SF43	Bjørkjenes	<i>Sphagnum fallax</i>	<i>Bacillus licheniformis</i> CP000002.2	98	Firmicutes	-	+
128	K	N2SM11	Sørdalen	<i>Sphagnum magellanicum</i>	<i>Bacillus licheniformis</i> AY842871.1	99	Firmicutes	-	+
129	K	N3SM25	Seljestad	<i>Sphagnum magellanicum</i>	ND			-	++

Table 3. Continued.

No.	ARDRA group*	Strain <sup>†</sup>	Origin		Closest database match and accession number	SI <sup>‡</sup>	Taxonomic grouping	Activity <sup>§</sup> against:	
			Location	Microenvironment				<i>V. d.</i>	<i>R. s.</i>
130	L	G3SF34	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	<i>Achromobacter</i> sp. AY170848.1	98	Beta-Proteobacteria	+	+
131	L	G3SF40	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	ND			+	–
132	L	G1SM24	Schlichtes Moor	<i>Sphagnum magellanicum</i>	<i>Achromobacter</i> sp. AY170848.1	99	Beta-Proteobacteria	–	+
133	L	G1SM34	Schlichtes Moor	<i>Sphagnum magellanicum</i>	ND			–	+
134	M	G2SF38	Dänschenburger Moor	<i>Sphagnum fallax</i>	<i>Pseudomonas</i> sp. AM042708.1	98	Gamma-Proteobacteria	–	+
135	M	G3SF31	Ribnitzer Großes Moor	<i>Sphagnum fallax</i>	ND			+	+
136	M	N1SF31	Bjorkjenes	<i>Sphagnum fallax</i>	ND			–	+
137	N	G3SM41	Ribnitzer Großes Moor	<i>Sphagnum magellanicum</i>	<i>Rahnella aquatilis</i> AY253919.1	98	Enterobacteria	++	+
138	N	N1SM27	Bjorkjenes	<i>Sphagnum magellanicum</i>	ND			–	++
139	N	N1SM35	Bjorkjenes	<i>Sphagnum magellanicum</i>	ND			–	++
140	O	N1SF22	Bjorkjenes	<i>Sphagnum magellanicum</i>	ND			–	+
141	O	G2SM2	Dänschenburger Moor	<i>Sphagnum magellanicum</i>	<i>Micrococcus</i> sp. AF408991.1	98	Actinobacteria	–	+
142	O	G3SM12	Ribnitzer Großes Moor	<i>Sphagnum magellanicum</i>	<i>Micrococcus luteus</i> AY167858.1	98	Actinobacteria	–	+
143	P	G3SM7	Ribnitzer Großes Moor	<i>Sphagnum magellanicum</i>	<i>Burkholderia thailandensis</i> AY268183.1	98	Beta-Proteobacteria	+	+
144	P	N1SM19	Bjorkjenes	<i>Sphagnum magellanicum</i>	<i>Burkholderia</i> sp. AJ971350.1	98	Beta-Proteobacteria	+	–
145	Q	N1SM15	Bjorkjenes	<i>Sphagnum magellanicum</i>	<i>Chromobacterium</i> sp. AY117572.1	98	Beta-Proteobacteria	+	–
146	Q	N2SM15	Sørdalen	<i>Sphagnum magellanicum</i>	<i>Chromobacterium</i> sp. AY117569.1	98	Beta-Proteobacteria	–	++
147	R	N1SF19	Bjorkjenes	<i>Sphagnum fallax</i>	<i>Arthrobacter koreensis</i> AY116497.1	99	Actinobacteria	–	+
148	R	N1SF26	Bjorkjenes	<i>Sphagnum fallax</i>	<i>Arthrobacter koreensis</i> AY116497.1	99	Actinobacteria	–	+
149	S	N1SF24	Bjorkjenes	<i>Sphagnum fallax</i>	<i>Pantoea agglomerans</i> AY691543.1	98	Enterobacteria	–	+
150	T	N2SF2	Sørdalen	<i>Sphagnum fallax</i>	<i>Micrococcus</i> sp. AF408991.1	98	Actinobacteria	+	–
151	U	N3SF34	Seljestad	<i>Sphagnum fallax</i>	<i>Plantibacter agrosticola</i> AF465411.1	98	Actinobacteria	–	+
152	V	G2SF19	Dänschenburger Moor	<i>Sphagnum fallax</i>	<i>Fulvimonas soli</i> AJ311653.1	97	Gamma-Proteobacteria	–	+
153	W	G1SF31	Schlichtes Moor	<i>Sphagnum fallax</i>	<i>Dyella japonica</i> AB110498.1	99	Gamma-Proteobacteria	–	+
154	X	G3SM33	Ribnitzer Großes Moor	<i>Sphagnum magellanicum</i>	<i>Delftia acidovorans</i> AF149849.1	99	Beta-Proteobacteria	+	–
155	Y	N1SF35	Bjorkjenes	<i>Sphagnum fallax</i>	<i>Yersinia kristensii</i> AJ627595.1	98	Enterobacteria	–	+

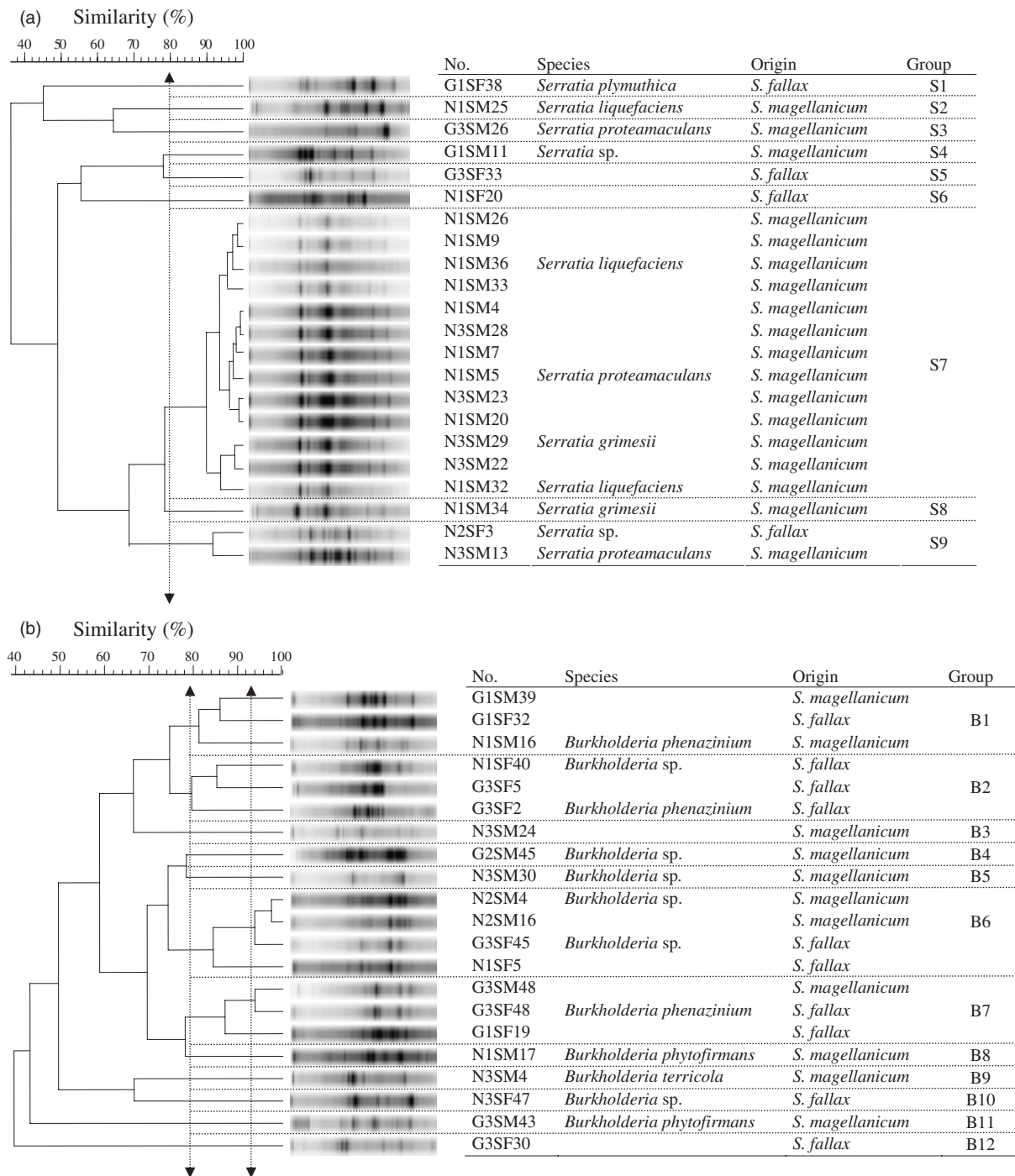
\*The letters represent the different ARDRA patterns (A–Y) of the 16S rRNA gene using HhaI.

<sup>†</sup>Letters represent the locations and microhabitats. G, Germany; N, Norway; SF, *Sphagnum fallax*; SM, *Sphagnum magellanicum*; arabic numerals represent the sampling site (1–3), and the strain number (1–50).

<sup>‡</sup>SI, similarity index: for isolates identified by 16S rRNA gene sequencing ranging from 0% to 100%.

<sup>§</sup>Antagonism towards *V. d.* (*Verticillium dahliae*) and *R. s.* (*Rhizoctonia solani*) was determined by dual-culture assay: +, represents 1–5 mm wide zone; ++, represents 5–10 mm wide zone; +++, represents 10–15 mm wide zone; –, represents no inhibition zone.

ND, not determined.



**Fig. 2.** Dendrogram showing the relationship of (a) the *Serratia* isolates from the ARDRA group A, and (b) the *Burkholderia* isolates from the ARDRA group C from *Sphagnum fallax* (SF) and *Sphagnum magellanicum* (SM) based on BOX-PCR fingerprints using cluster analysis by the unweighted pair group method and arithmetic averages. Double-headed vertical arrows indicate the similarity for the groupings.

## Discussion

*Sphagnum* plants form unique host plants for microorganisms and are known for their antimicrobial activity. Interestingly, no *Sphagnum*-specific pathogenic fungi are known.

Many chemical compounds which are currently largely unknown, until now, are responsible for this antimicrobial activity (Asakawa & Heidelberger, 1982; Frahm, 2001). It is also of interest to determine whether *Sphagnum* plants harbour antagonistic bacteria that take part in the

antimicrobial activity. In our study, a very high proportion of antagonistic bacteria was found for both *Sphagnum* species (*S. magellanicum*: 24%; *S. fallax*: 19%). The highest proportion of antagonistic bacteria reported in the literature was found for *Sphagnum rubellum* (31%, Opelt & Berg, 2004), whereas 3–9% was found in the rhizosphere of *Verticillium* host plants (Berg et al., 2002), 16% in the rhizosphere of oilseed rape (Berg, 1996), and 18% in the rhizospheres of various weeds (Kremer et al., 1990). It is known that fungi (including those with pathogenic properties) prefer habitats with acidic conditions. Effective defence strategies against pathogenic fungi are therefore essential for *Sphagnum* plants that occur in habitats at low pH. The powerful antifungal bacteria found in this study provide a hint that bacteria are involved in the defence strategy against fungi, as shown for bacteria living in the rhizosphere (Cook et al., 1995; Weller et al., 2002). However, the high proportion of antagonistic bacteria is surprising when the rhizosphere effect is taken into consideration. This is the phenomenon that, in comparison to that in other plant-associated microenvironments or in the bulk soil, the number of microorganisms in the rhizosphere is enhanced (Lynch, 1990; Sørensen, 1997), and those with antagonistic properties are enriched because of the rich exudation of roots (Berg et al., 2002; 2006). *Sphagnum* plants have no roots and no exudation of nutrients is known. However, *Sphagnum* plants have the ability to release hydrogen ( $H^+$ ) ions in exchange for dissolved cations (Andrus, 1986). Bryophytes have a high capacity for cation exchange, with more cation-binding sites per unit area of cell wall than any other plant (Gignac, 1987). This process alters the pH of *Sphagnum* surroundings and acidifies the *Sphagnum* habitat. The plant also modifies its environment considerably by raising the peat surface. *Sphagnum* has the ability to absorb large amounts of water, usually 10 to 20 times its dry weight (Andrus, 1986). In conclusion, *Sphagnum* gametophytes can absorb water and dissolved minerals over their surfaces, and this may be one reason for bacterial colonization inside and outside. As a result of the experimental procedure of our study we analysed endophytic as well as ectophytic bacteria of *Sphagnum*. By analysing both microenvironments separately, we determined that they are both colonized by highly diverse populations (unpublished data).

Higher proportions of antifungal isolates were found for *S. magellanicum* than for *S. fallax*. The ecological behaviour of the two *Sphagnum* species is different, and may be one reason for this difference. For *S. magellanicum*, habitat conditions with extreme acidity (reaction value: 1.3–1.7) and extreme nutrient poorness (nutrient value 1.0–1.6) were found (Table 1). In contrast, for the habitats of *S. fallax* the reaction values ranged between 2.3 and 3.0, and the nutrient values between 1.8 and 3.1. Whereas *S. magellanicum* is typical for strongly acidic and oligotrophic habitats,

*S. fallax* grows in weakly acidic, mesotrophic situations. For both *Sphagnum* species we found strongly varying proportions of antagonists against *V. dahliae* as well as against *R. solani* regarding the two different geographical sites. Whereas for *S. magellanicum* the highest proportion of antagonistic isolates was found for the samples from Norway, the opposite was found for bacteria associated with *S. fallax*: here we found a very high proportion of antagonists at the German sampling sites. During the bryophyte sampling in the various peat bogs of Germany and Norway it was remarkable that *S. fallax* was frequent at the German sites but rare in Norway, whereas *S. magellanicum* was frequent at the Norwegian sites but rare in Germany. The two *Sphagnum* species prefer different habitats and were dominantly found in bogs that provided optimal growth conditions in each case. The displacement of other *Sphagnum* species and the increasing distribution of *S. fallax* is a typical phenomenon for disrupted bogs. Many bogs are affected by atmospheric pollution and nutrient enrichment by agricultural fertilizer (Lüdtke-Twenhöven, 1992). This alters the nutrient status of the bog, and hence the plant-species composition.

The majority of all selected antagonistic bacteria showed an activity against *R. solani* (87%), whereas only 53% of the antagonists showed an activity against *V. dahliae*. The antagonistic activity against *R. solani* or *V. dahliae* was specific for each isolate. Different mechanisms of antagonistic activity and different targets of the pathogen may be responsible for this. In addition, the phytopathogenic fungi *R. solani* has the ability to survive as sclerotia under adverse soil environmental conditions for many years (Ogoshi, 1996). Its ability to engage in parasitic as well as in saprophytic activity makes possible the colonization of an extremely wide host and habitat range. Interestingly, antagonistic isolates of the genera *Pseudomonas* (ARDRA group D), *Micrococcus* (ARDRA group O), and *Arthrobacter* (ARDRA group R) were active against *R. solani*, exclusively. Furthermore the isolates of the ARDRA group K, which were identified as *Bacillus*, showed an antifungal activity against *R. solani* but no activity against *V. dahliae*. The majority of the isolates of the ARDRA groups A, B, and C, which are represented by the genera *Serratia*, *Staphylococcus* and *Burkholderia*, showed an activity against both pathogenic fungi. According to our results, it is conceivable that, in addition to chemical contents with antimicrobial activity, the *Sphagnum*-associated microorganisms may also take part in the pathogen defence.

The characterization of the antagonistic isolates by 16S rRNA gene RFLP resulted in 25 *Sphagnum* species-specific ARDRA groups. Antagonists from *S. magellanicum* were dominated by the genus *Serratia* (ARDRA group A), whereas the genus *Pseudomonas* (ARDRA group D) was dominantly found for *S. fallax*. However, the genera

*Staphylococcus* (ARDRA group B) and *Burkholderia* (ARDRA group C) were also found as dominant and important genera with antagonistic abilities for *S. fallax* as well as for *S. magellanicum*. The genus *Serratia* has been dominantly found for antagonistic bacteria of *S. magellanicum* in previous studies (unpublished data). In addition, for *Serratia*-specific communities a higher genetic diversity was found for *S. magellanicum* than for *S. fallax* using cultivation-independent analysis (unpublished data). Using BOX-PCR we found a high genotypic diversity and plant specificity at the genotypic level for isolates of the genus *Serratia*. However, we found isolates of the genus *Serratia* with the same genotype on *S. fallax* and on *S. magellanicum*. While *Pseudomonas* and *Serratia* are well-known antagonistic genera (Berg, 2000; Lugtenberg *et al.*, 2001; Graner *et al.*, 2003; Mercado-Blanco *et al.*, 2004), *Staphylococcus* is an interesting but rarely mentioned genus. *Burkholderia* strains have been shown to be antagonistic and plant-growth-promoting bacteria (Burkhead *et al.*, 1994; Tran Van *et al.*, 2000; Estrada-De Los Santos *et al.*, 2001; Quan *et al.*, 2005; Sessitsch *et al.*, 2005). The antagonistic activity of *Sphagnum*-associated *Burkholderia* strains against *V. dahliae* was shown by Opelt & Berg (2004). Two of the isolates could be identified as *Burkholderia phytofirmans*, which is known for its antagonistic and plant-growth-promoting activity (Sessitsch *et al.*, 2005). Six isolates (G2SM45, N1SF40, N3SF47, N1SM19, N2SM4, and N3SM30) were phylogenetically close (97–99% identity of 16S rRNA gene sequences) to the strain *Burkholderia* sp. Y (AJ971350.1), which was isolated from the peat of the Risti bog in Estonia, and one isolate (G3SF45) showed 99% identity of the 16S rRNA gene sequence to the strain *Burkholderia* sp. SB1 (AJ971347.1), which was isolated from the peat of the Kurovskoe bog, Moscow oblast (Belova *et al.*, 2006). This underlines the high specificity of *Sphagnum*-associated bacteria.

Our results show that *Sphagnum* species other than *Sphagnum rubellum* are natural ecological niches for bacteria of the genus *Burkholderia* (Opelt & Berg, 2004), and confirm the studies of Belova *et al.* (2006), which showed that bacteria of the genus *Burkholderia* are typical components of the microbial community of *Sphagnum* peat bogs. Belova *et al.* (2006) showed that *Burkholderia* isolates are moderately acidophilic, psychrotolerant, dinitrogen-fixing microorganisms well adapted to the conditions of northern acidic *Sphagnum* bogs. Characteristic for all *Burkholderia* strains is their ability to use a wide range of organic compounds as carbon and energy sources (Yabbuchi *et al.*, 1992). The physiological characteristics of this genus allow it to colonize a variety of habitats, such as soil, plants, animals, rhizosphere, and water; the habitats show partly extreme conditions (Parke & Gurian-Sherman, 2001; Coenye & Vandamme, 2003; Ramette *et al.*, 2005; Sessitsch *et al.*,

2005). In addition to the isolates of the genus *Serratia*, we also found a high genotypic diversity and plant-specific genotypes for the isolates of the genus *Burkholderia*. Nevertheless, for the isolates G3SM38 and G3SF48 the same genotype was found on different *Sphagnum* species. Interestingly, for the isolates N2SM4, N2SM16 and G3SF45 the same genotype was found not only on different *Sphagnum* species but also at different geographical sites. In addition to well-known antagonistic genera like *Serratia*, *Bacillus*, and *Pseudomonas*, which are typical for different crop plants (Krechel *et al.*, 2002; Faltin *et al.*, 2004; Sessitsch *et al.*, 2004; Berg *et al.*, 2006), we found a high proportion of *Sphagnum*-associated antagonists that belonged to extraordinary genera such as *Chromobacterium*, *Hafnia*, *Achromobacter*, *Arthrobacter*, *Fulvimonas*, *Dyella*, *Delftia*, *Micrococcus*, and *Plantibacter*.

Furthermore, many strains were detected that are known as facultative or opportunistic pathogens of humans, which cause diseases only in patients with a strong predisposition to illness, particularly in those who are severely debilitated, immunocompromised or suffering from cystic fibrosis or HIV-infections. They are grouped into risk group 2 in the public databases, for example those by the German Collection of Microorganisms and Cell Cultures ([www.dsmz.de](http://www.dsmz.de)), such as *Pantoea agglomerans*, *Yersinia kristensii*, *Hafnia alvei*, *Burkholderia multivorans*, *Rothia amarae*, *Staphylococcus pasteurii*, *Staphylococcus caprae*, and *Staphylococcus epidermidis*. These results strongly suggest that the bryophyte genus *Sphagnum* is a reservoir for potentially facultative human pathogens. In the last two decades, the impact of opportunistic infections on human health has increased dramatically. With advances in medical technology, and the growth of at-risk populations, the incidence of infections caused by opportunistic pathogens is expected to increase further. Knowledge about their natural reservoirs is therefore important. The rhizosphere is known as one natural reservoir of opportunistic human pathogens (Berg *et al.*, 2005). Many genera, for example *Burkholderia*, *Enterobacter*, *Ochrobactrum*, *Pseudomonas*, *Staphylococcus* and *Stenotrophomonas*, contain root-associated bacteria that enter bivalent interaction with plant and human hosts. Mechanisms involved in the interaction between beneficial plant-associated bacteria and their host plants are similar to those responsible for the pathogenicity of bacteria (Rahme *et al.*, 1995). These mechanisms may also be involved in colonizing the human body.

In conclusion, *Sphagnum* plants form an extreme habitat for microorganisms but they are colonized by specific bacterial populations that are adapted to these special conditions. The high recovery of antagonistic isolates strongly suggests that *Sphagnum* mosses harbour antifungal bacteria, which take part in the pathogen defence. The antifungal bacteria as well as the *Sphagnum* plantlets

themselves could be a source for natural fungicides. Moreover, *Sphagnum* plants represent an interesting tool for understanding the natural self-protection of plants and for the detection of new antagonists that could form a basis for biocontrol. They represent an ecological niche not only for diverse and extraordinary microbial populations with a high potential for the biological control of plant pathogens, but also for potentially facultative human pathogens.

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