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Bacillus and *Streptomyces* were selected as broad-spectrum antagonists against soilborne pathogens from arid areas in Egypt

Martina Köberl[1], Elshahat M. Ramadan[2], Mohamed Adam[3], Massimiliano Cardinale[1], Johannes Hallmann[3], Holger Heuer[3], Kornelia Smalla[3] & Gabriele Berg[1]

[1]Institute for Environmental Biotechnology, Graz University of Technology, Graz, Austria; [2]Faculty of Agriculture, SEKEM, Heliopolis University, Ain Shams University, Cairo, Egypt; and [3]Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut – Federal Research Centre for Cultivated Plants, Braunschweig, Germany

Correspondence: Gabriele Berg, Institute for Environmental Biotechnology, Graz University of Technology, Petersgasse 12/I, A-8010 Graz, Austria. Tel.: +43 316 873 8310; fax: +43 316 873 8819; e-mail: gabriele.berg@tugraz.at

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Abstract

Plant protection via disease-suppressive bacteria in desert farming requires specific biological control agents (BCAs) adapted to the unique arid conditions. We performed an ecological study of below-ground communities in desert farm soil and untreated desert soil, and based on these findings, selected antagonists were hierarchically evaluated. In contrast to the highly specific 16S rRNA fingerprints of bacterial communities in soil and cultivated medicinal plants, internal transcribed spacer profiles of fungal communities were less discriminative and mainly characterised by potential pathogens. Therefore, we focused on *in vitro* bacterial antagonists against pathogenic fungi. Based on the antifungal potential and genomic diversity, 45 unique strains were selected and characterised in detail. *Bacillus*/*Paenibacillus* were most frequently identified from agricultural soil, but antagonists from the surrounding desert soil mainly belonged to *Streptomyces*. All strains produced antibiotics against the nematode *Meloidogyne incognita*, and one-third showed additional activity against the bacterial pathogen *Ralstonia solanacearum*. Altogether, 13 broad-spectrum antagonists with antibacterial, antifungal and nematicidal activity were found. They belong to seven different bacterial species of the genera *Bacillus* and *Streptomyces*. These Gram-positive, spore-forming bacteria are promising drought-resistant BCAs and a potential source for antibiotics. Their rhizosphere competence was shown by fluorescence *in situ* hybridisation combined with laser scanning microscopy.

Introduction

While desertification is recognised as a major threat to biodiversity, the conversion of desert soil into arable, green landscapes is a global vision (Clery, 2011; Marasco *et al.*, 2012). Desert farming, which generally relies on irrigation, is one way to potentially realise this goal. In Australia, Israel, California and Africa, desert farming areas are expanding. For example, desert farming in Egypt will have grown by 40% by 2017 (Reuters, 2007). However, emerging problems with soilborne pathogens, which can substantially limit crop yield, are often reported after several years of agricultural land use (Krikun *et al.*, 1982). These soilborne pathogens include various taxonomic groups, for example, fungi (*Fusarium culmorum*, *Rhizoctonia solani*, *Verticillium dahliae*), bacteria (*Ralstonia solanacearum*) and nematodes (*Meloidogyne incognita*) (Klosterman *et al.*, 2009; Messiha *et al.*, 2009; Neher, 2010). Because of its depleting effect on the ozone layer, the extensively used broad-spectrum soil fumigant methyl bromide was banned by the Montreal Protocol in 1987 and phased out in most countries by 2005. Now, there is an urgent demand for ecologically compatible and efficient strategies to suppress soilborne pathogens in both conventional and organic desert agriculture (Bashan & de-Bashan, 2010).

Biological control based on naturally occurring antagonists offers sustainable solutions for plant protection (Weller, 2007; Berg, 2009; Lugtenberg & Kamilova, 2009; Raaijmakers *et al.*, 2009). However, beneficial plant–microorganism interactions are highly specific, and only a few broad-spectrum antagonists have been reported (Zachow *et al.*, 2008; Hartmann *et al.*, 2009). Gram-negative bacteria, especially those from genus *Pseudomonas*, were identified as the dominant members of the indigenous antagonistic communities under humid conditions (Berg *et al.*, 2005; Haas & Défago, 2005; Costa *et al.*, 2006; Zachow *et al.*, 2008) and as a major group of disease-suppressive bacteria through pyrosequencing (Mendes *et al.*, 2011). Although there are problems with the formulation and shelf life of *Pseudomonas*, strains have still been developed as commercial BCAs (Weller, 2007; Berg, 2009). Gram-positive bacteria have also been widely used as BCAs and plant growth-promoting rhizobacteria (PGPRs), even though their ability to colonise the rhizosphere has been controversial (Hong *et al.*, 2009; Fan *et al.*, 2011). Their ability to form durable, heat-resistant endospores allows for easy formulation (Emmert & Handelsman, 1999; Adesemoye *et al.*, 2009), but their use as BCAs in desert agroecosystems is not been established so far.

Desert soils are characterised by arid conditions, which include a combination of extreme temperatures and desiccation, high soil salinity, low nutrient levels, high UV radiation levels and physical instability caused by strong winds (Cary *et al.*, 2010). In one of the most prominent examples of organic desert farming in Sekem (Egypt), we found a strong correlation between long-term organic agriculture and bacterial community composition in soils. Bacterial communities in agricultural soil showed a higher diversity and a better ecosystem function for plant health compared to the surrounding natural desert soil (Köberl *et al.*, 2011). A comprehensive analysis explained these structural differences: the proportion of *Firmicutes* represented by antagonistic *Bacillus* and *Paenibacillus* in field soil was significantly higher (37%) than in the desert soil (11%). In contrast, *Actinobacteria* occurred in farmland in lower concentrations (5%) than in the desert (21%), and antagonistic isolates of *Streptomyces* were only isolated from native desert soil (Köberl *et al.*, 2011). A high presence of *Actinobacteria* in soil of the North American Sonoran Desert was also found by 454-pyrotag analyses (Andrew *et al.*, 2012) as well as in soil of the hyperarid Atacama Desert in north-west Chile (Neilson *et al.*, 2012). From the latter, several so far unknown *Streptomyces* spp. were recently described (Santhanam *et al.*, 2012a,b, 2013). In addition, a study examining soil bacterial communities in the Negev Desert in the south of Israel even revealed a higher abundance of *Actinobacteria* in barren soils compared to soils under shrub canopies (Bachar *et al.*, 2012). However, the indigenous desert microbiome should contain BCAs that are adapted to the specific biotic and abiotic conditions of desert habitats as well as strains that produce novel bioactive compounds, because the genus *Streptomyces* is known as a unique source of novel antibiotics (Goodfellow & Fiedler, 2010; Niraula *et al.*, 2010; Nachtigall *et al.*, 2011). The potential for both has been until now poorly understood and used.

The objective of this study was to analyse microbial communities from agricultural desert habitats (e.g. from the rhizospheres and endorhiza) in comparison with the surrounding desert soil for their biocontrol potential and to specifically select and characterise broad-spectrum antagonists against soilborne pathogens regarding this potential.

Materials and methods

Experimental design and sampling

Microbial diversity in organic desert farming was studied at Sekem farms (www.sekem.com) in Egypt (30°22′88″N, 31°39′41″E) in comparison with surrounding desert soil (30°35′01″N, 32°25′49″E; 35°59′0″N, 41°2′0″E). The sampling strategy is described in detail in Köberl *et al.* (2011). Briefly, at each site, four composite samples of soil in a horizon of 0–30 cm depth were collected. Furthermore, roots with adhering soil were obtained from three different species of medicinal plants (*Matricaria chamomilla* L., *Calendula officinalis* L. and *Solanum distichum* Schumach. and Thonn.) planted on a Sekem farm. From each plant species, four independent composite samples consisting of 5–10 plants were taken. Samplings were performed in October 2009 and in April 2010. Physico-chemical data of the soil are provided in Luske & van der Kamp (2009).

Microbial fingerprints from single-stranded conformational polymorphism analysis of the ITS and 16S rRNA region (PCR-SSCP)

Total community DNA was isolated from bulk soil, rhizosphere and endorhiza of the medicinal plants according to Köberl *et al.* (2011). Fingerprinting of microbial communities by SSCP was performed as described by Schwieger & Tebbe (1998). Amplification of the fungal internal transcribed spacer (ITS) fragment was performed by a nested PCR approach with primer pairs ITS1/ITS4 and ITS1/ITS2^{P} (White *et al.*, 1990). Nested PCR was performed as described by Zachow *et al.* (2008). SSCP analysis of bacterial 16S rRNA gene sequences is specified in Köberl *et al.* (2011). Sequences of excised and re-amplified

bands were submitted to EMBL Nucleotide Sequence Database under accession numbers FR854281-FR854290, FR871639-FR871646 and HE655458-HE655480.

SSCP profiles of the microbial communities generated with universal fungal and bacterial primers were further applied for multivariate analysis. According to the distance of the bands, the SSCP gels were theoretically divided into operational taxonomic units (OTUs). The presence or absence of individual amplified product DNA bands in each group was scored. OTUs served as response variables for principal component analysis (PCA) using Canoco 4.5 for Windows (Lepš & Smilauer, 2003). Matrices based on Pearson correlation were subjected to significance tests of pairwise similarities by applying permutation analyses ($P < 0.05$) using the permtest package of R statistics version 2.13.1 (The R Foundation for Statistical Computing, Vienna, Austria) with 10^5 random permutations of sample elements (Kropf *et al.*, 2004; R Development Core Team, 2011).

Screening for *in vitro* activity against soilborne bacteria and nematodes

Forty-five promising strains with antagonistic activity against pathogenic fungi (Köberl *et al.*, 2011) were tested for antibacterial activity against *Ralstonia solanacearum* 1609 and B3B. The activity of all isolates against both *R. solanacearum* strains was identical; therefore, the data in Table 2 are presented in singular form. For the screening, yeast peptone glucose (YPG) medium was used, and Tetrazolium Violet (Sigma-Aldrich, Saint Louis, USA) was added to the medium prior to pouring as a redox indicator of bacterial growth (Adesina *et al.*, 2007; Tsukatani *et al.*, 2008).

For testing the activity of the selected antagonists towards the phytopathogenic nematode *Meloidogyne incognita* (Kofoid and White) Chitwood, culture supernatants from the bacteria were prepared. For this, the bacterial isolates were grown at 28 °C for 24 h on R2A agar (Merck, Darmstadt, Germany). A preculture was grown over night from a single colony in 5 mL of tryptic soy broth (TSB) (Merck) with 50 mg L^{-1} rifampicin at 28 °C with shaking at 150 r.p.m. 200 μL of the preculture were added to 100 mL sterile TSB and incubated for 24 h at 28 °C with shaking. The bacteria were then removed from the culture by centrifugation at 7500 ***g*** for 20 min, followed by sterile filtration of the supernatants through membranes with 0.22 μm pore size. The sterile culture supernatants were kept at 4 °C until application. To study the effect of extracellular bacterial products on the mortality of *M. incognita* juveniles (J2), 500 μL of a juvenile suspension containing approximately 100 freshly hatched J2 was mixed with 1 mL of each bacterial filtrate in a Petri dish with 500 μL of an antibiotic solution containing 300 mg L^{-1} streptomycin and 300 mg L^{-1} penicillin to suppress microbial growth. Each treatment was replicated 4 times. Controls consisted of TSB, water and a culture supernatant of the nonantagonistic strain *Escherichia coli* JM109, respectively. All dishes were kept at 25 ± 2 °C in the dark. Numbers of motile and nonmotile nematodes were counted after 6, 12, 24 and 48 h using a binocular microscope. To distinguish between nonmotile and dead J2, the nematodes were transferred to water at the end of the exposure time. Juveniles that did not recover and become motile again were considered dead. The rate of mortality was determined using linear regression of the percentages of dead J2 after 0, 6, 12 and 24 h.

Fluorescence *in situ* hybridisation (FISH) and confocal laser scanning microscopy (CLSM)

Samples were fixed in 4% paraformaldehyde and stained by in-tube FISH according to the protocol of Cardinale *et al.* (2008). An equimolar mixture of Cy3-labelled EUB338, EUB338II and EUB338III probes (Amann *et al.*, 1990; Daims *et al.*, 1999) was used for the detection of all bacteria and a Cy5-labelled HGC236 probe (Erhart *et al.*, 1997) for the detection of *Actinobacteria*. As a negative control, nonsense FISH probes labelled with both fluorochromes (NONEUB; Wallner *et al.*, 1993) were applied. Confocal images were obtained using a Leica TCS SPE confocal laser scanning microscope (Leica Microsystems GmbH, Mannheim, Germany).

Results

Molecular fingerprinting of microbial belowground communities

All investigated SSCP fingerprints of the ITS and 16S rRNA gene fragments from both the rhizosphere and endorhiza of the medicinal plants and bulk soil showed a high diversity. According to the statistical cluster analysis, there is a clear plant-specific effect on both communities in the rhizosphere (Fig. 1, Table 1). Furthermore, microenvironment-specific SSCP patterns of the microbial communities were detected, and statistically significant differences between the rhizosphere and the endorhiza of the medicinal plants were calculated (Fig. 1, Table 1). Additionally, plant-associated microenvironments were compared with the surrounding soil. The composition of the bacterial and fungal communities in soil differed significantly from the plant-associated communities (*P* values: fungal communities 0.0241; bacterial communities 0.0266) and between agricultural and desert soil (*P* values: fungal communities 0.0291; bacterial communities 0.0289).

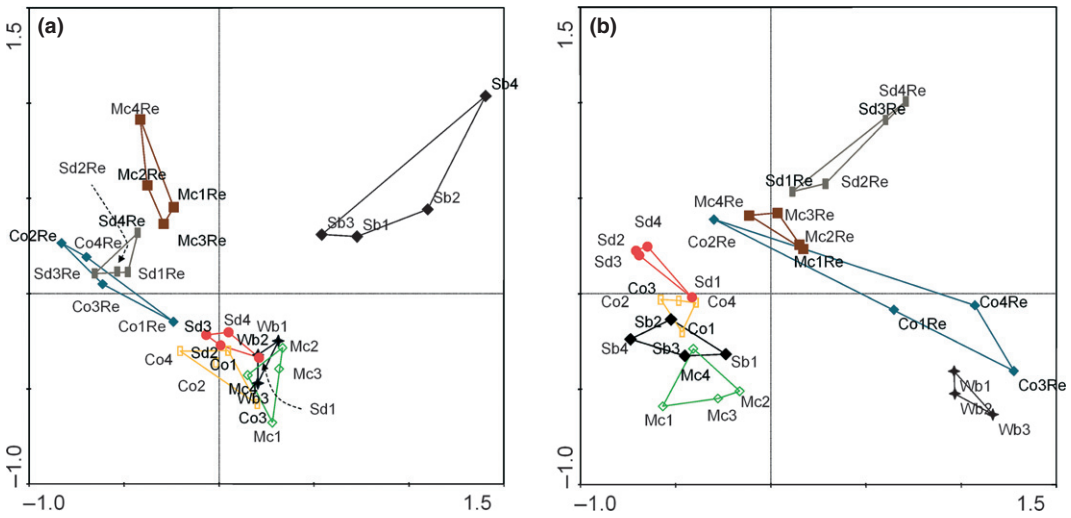

Fig. 1. PCA of OTUs identified by SSCP fingerprinting for fungal (a) and bacterial (b) communities. Samples were encoded using a combination of letters and numbers indicating (1) soil type or plant species (Wb = desert soil, Sb = Sekem soil, Mc = *Matricaria chamomilla*, Co = *Calendula officinalis*, Sd = *Solanum distichum*), (2) replicate (1–4) and (3) microenvironment (Re = endorhiza, rhizosphere and soil have no further designation).

Table 1. Statistical analysis of microbial fingerprints obtained by PCR-SSCP.

	Fungal communities	Bacterial communities
Microenvironment	*P* values for pairwise comparisons between medicinal plants*	
Rhizosphere†		
Mc-Co	0.0276	0.0281
Co-Sd	0.0284	0.0286
Mc-Sd	0.0296	0.0286
Endorhiza†		
Mc-Co	0.0297	0.0556
Co-Sd	0.0719	0.0283
Mc-Sd	0.0282	0.0293
Medicinal plant	*P* values for comparisons between rhizosphere and endorhiza*	
Matricaria chamomilla	0.0290	0.0287
Calendula officinalis	0.0288	0.0287
Solanum distichum	0.0287	0.0281

*Analysed by permutation test ($P < 0.05$) using R statistics.
†Mc, *Matricaria chamomilla*; Co, *Calendula officinalis*; Sd, *Solanum distichum*.

The fingerprints of the fungal community represented a high diversity in all microenvironments and were similar for the first and second samplings (Fig. 2). In general, potential plant pathogens were frequently found within the fungal communities. In fingerprints from both samplings, *Alternaria* (closest database match *Alternaria tenuissima*, 100% similarity to JN620417) and *Fusarium* (closest database matches *Fusarium chlamydosporum*, 100% similarity to HQ671187 and *Fusarium solani*, 99% similarity to FJ865435) were most commonly found. *Alternaria* was also found in desert soil from Sinai (first sampling) as well as from Saqqara (second sampling). In addition, *Cladosporium* (teleomorph *Davidiella*) was identified in fingerprints from both samplings. In rhizosphere and soil samples from the first sampling, *Epicoccum* (closest database match *Epicoccum nigrum*, 100% similarity to JN578611) was assigned to a dominant band. In soil from the Sinai desert, the black fungus *Aureobasidium* (closest database match *Aureobasidium proteae*, 99% similarity to JN712490) was additionally identified. Similarly, *Verticillium dahliae* (closest database match *V. dahliae* var. *longisporum*, 100% similarity to AB585937) was identified as a dominant band found in almost all plant samples from the second sampling time, which apart from *Fusarium* spp. was one of the main soilborne phytopathogens on the Sekem farms. In samples from the second sampling, the obligate root-infecting pathogen *Olpidium* (closest database match *Olpidium brassicae*, 99% similarity to AB625456), belonging to the fungal phylum Chytridiomycota, and *Sarocladium* (closest database match *Sarocladium strictum*, 100% similarity to JN942832; previously recognised in *Acremonium*) were found. Although several other ITS fragments were not identified, due to this high content of potential phytopathogens in the fungal communities, the selection of antagonists was focused on the bacterial communities.

Detailed characterisation of selected antagonistic strains

A screening of 1212 bacterial isolates resulted in 162 antifungal antagonists against the main fungal soilborne

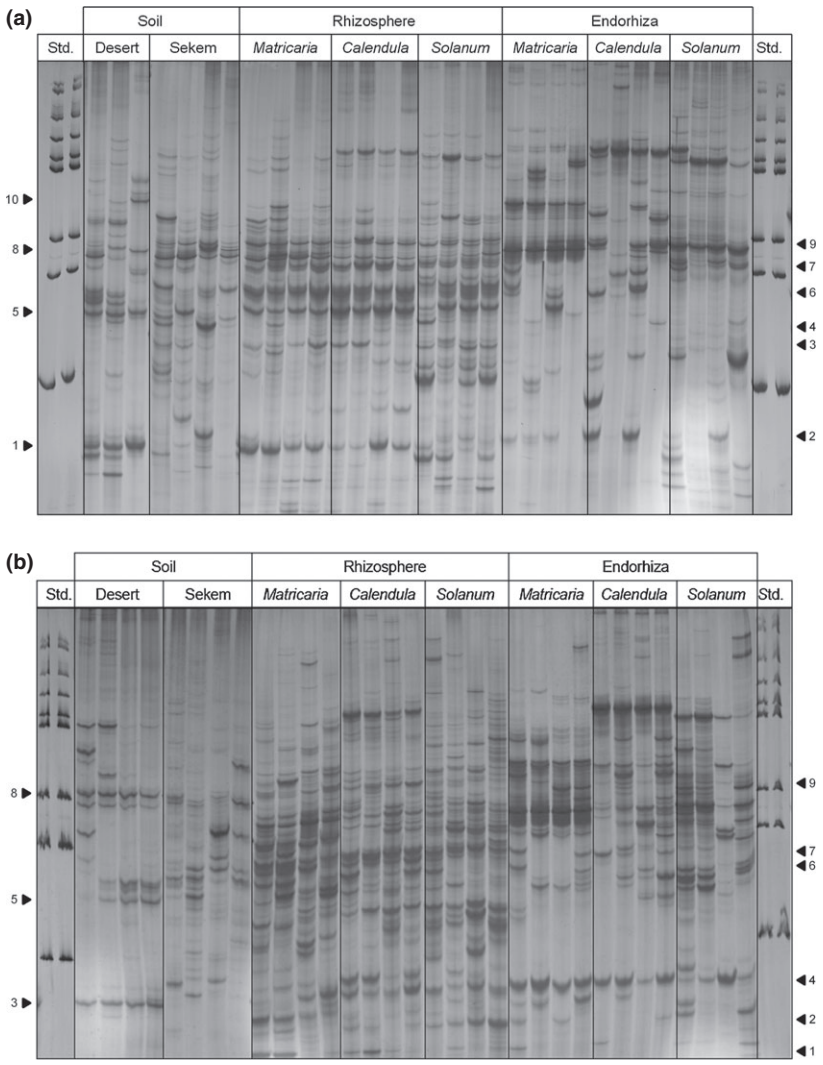

Fig. 2. ITS PCR-SSCP profiles of the fungal communities in soil, rhizosphere and endorhiza of the medicinal plants from first (a) and second (b) sampling time. Std.: 1 kb DNA ladder. (a) From fingerprints of the first sampling (October 2009), the following bands were identified as: 1. *Epicoccum nigrum*, 100% similarity to JN578611; 2. *Pichia jadinii*, 99% similarity to FJ865435; 3. *Gibellulopsis nigrescens*, 100% similarity to JN187998; 4. *Emericella nidulans*, 99% similarity to JN676111; 5. *Alternaria tenuissima*, 100% similarity to JN620417; 6. *Davidiella tassiana*, 99% similarity to JN986782; 7. *Fusarium chlamydosporum*, 100% similarity to HQ671187; 8. *Exserohilum rostratum*, 99% similarity to JN179081; 9. *Fusarium solani*, 99% similarity to FJ865435; 10. *Aureobasidium proteae*, 99% similarity to JN712490. (b) From the second sampling (April 2010), the following bands were identified: 1. *Cryptococcus carnescens*, 99% similarity to GU237051; 2. *Olpidium brassicae*, 99% similarity to AB625456; 3. *Preussia minimoides*, 96% similarity to AY510422; 4. *Verticillium dahliae* var. *longisporum*, 100% similarity to AB585937; 5. *Alternaria tenuissima*, 100% similarity to JN620417; 6. *Fusarium chlamydosporum*, 99% similarity to EU556725; 7. *Cladosporium cladosporioides*, 100% similarity to JN986781; 8. *Ulocladium oudemansii*, 100% similarity to FJ266488; 9. *Sarocladium strictum*, 100% similarity to JN942832.

pathogens (*V. dahliae*, *R. solani* and *F. culmorum*) (Köberl *et al.*, 2011). These fungi were identified in Sekem soil by cultivation and, with the exception of *R. solani*, in the molecular fingerprinting analyses. Altogether, 45 genotypically unique antifungal strains were selected to assess their antibacterial activity against *R. solanacearum* (Table 2). Of these isolates, 33.3% were able to inhibit the growth of the soilborne bacterial pathogen *in vitro*, including most isolates of *Streptomyces* (3 of 4 isolates) and some strains of the *Bacillus subtilis* group (12 of 30 isolates).

Plant-parasitic nematodes often positively interact with soilborne fungal pathogens. Therefore, the selected bacterial isolates were additionally evaluated *in vitro* for their effects against juveniles of the root-knot nematode *M. incognita*. All bacteria accumulated inhibitory substances in the culture medium to some degree, while the medium itself and water had no effect. The percentage of dead J2 continuously increased during the incubation period of 48 h reaching over 70% for 11 strains with a maximum of 89% for strain Mc5Re-2, while only 28% of J2 were dead in the *E. coli* control (Table 2). On average, the increase in mortality was highest within the first 12 h of exposure and declined thereafter. The ten most efficient strains caused between 47% and 63% mortality in the first 24 h, with the highest rates observed for strains Sb4-23, Mc5Re-2, Mc1Re-3 and Sb3-24 (Fig. 3). The seven most efficient antagonists were all isolates of *Bacillus subtilis* obtained from either agricultural soil or from the endorhiza of *M. chamomilla*.

In situ visualisation of *Actinobacteria* in the rhizosphere

FISH-CLSM analysis confirmed generally high bacterial abundances and occurrence of *Actinobacteria* in below-ground habitats under arid conditions. Using an

Table 2. List of selected bacterial antagonists isolated from different microenvironments with their antagonistic properties.

			Antagonistic activity towards‡					
							Meloidogyne incognita¶	
ARDRA group*	Isolate number	Closest database match† (accession number), similarity (%)	*Verticillium dahliae*§	*Rhizoctonia solani*§	*Fusarium culmorum*§	*Ralstonia solanacearum*	Dead J2 after 48 h (%)‖	Mortality rate (% J2 per day)**
A	Wb2n-1	*Bacillus vallismortis* (NR_024696), 99%	+	++	+	+	73 ± 6	49 ± 4
A	Sb1-6	*Bacillus subtilis* subsp. *subtilis* (NR_027552), 99%	+	–	+	+	54 ± 4	32 ± 2
A	Sb3-5	*Bacillus subtilis* subsp. *subtilis* (NR_027552, 99%	+	++	+	+	46 ± 3	25 ± 3
A	Sb3-13	*Bacillus atrophaeus* (NR_024689), 99%	+	++	+	+	33 ± 3	17 ± 1
A	Sb3-21	*Bacillus subtilis* subsp. *spizizenii* (NR_024931), 99%	+	++	+	–	68 ± 7	52 ± 4
A	Sb3-24	*Bacillus subtilis* subsp. *subtilis* (NR_027552), 99%	+	++	+	–	78 ± 7	57 ± 4
A	Sb4-14	*Bacillus vallismortis* (NR_024696), 99%	+	+	+	–	45 ± 5	23 ± 1
A	Sb4-23	*Bacillus subtilis* subsp. *subtilis* (NR_027552), 99%	+	+	+	+	84 ± 5	63 ± 3
A	Mc3-4	*Bacillus mojavensis* (NR_024693), 98%	+	++	++	+	67 ± 8	30 ± 2
A	Mc5-18	*Bacillus subtilis* subsp. *subtilis* (NR_027552), 99%	++	++	++	–	29 ± 2	14 ± 2
A	Mc5-19	*Bacillus subtilis* subsp. *subtilis* (NR_027552), 99%	+	+	–	+	35 ± 4	17 ± 2
A	Co1-6	*Bacillus subtilis* subsp. *subtilis* (NR_027552), 99%	++	++	++	+	70 ± 7	37 ± 3
A	Co2-14	*Bacillus subtilis* subsp. *spizizenii* (NR_024931), 99%	+	+	++	–	72 ± 12	40 ± 5
A	Co7-19	*Bacillus subtilis* subsp. *spizizenii* (NR_024931), 100%	++	+	+	–	48 ± 5	26 ± 1
A	Sd1-14	*Bacillus subtilis* subsp. *spizizenii* (NR_024931), 99%	+	++	++	–	56 ± 5	35 ± 3
A	Sd3-12	*Bacillus subtilis* subsp. *subtilis* (NR_027552), 100%	+	+	++	–	29 ± 2	17 ± 1
A	Sd3-21	*Bacillus subtilis* subsp. *spizizenii* (NR_024931), 99%	+	++	+	–	57 ± 4	35 ± 5
A	Sd7-15	*Bacillus subtilis* subsp. *spizizenii* (NR_024931), 100%	+	++	+	–	43 ± 4	26 ± 2
A	Mc1Re-3	*Bacillus subtilis* subsp. *subtilis* (NR_027552), 99%	+	++	++	–	80 ± 4	56 ± 7
A	Mc2Re-2	*Bacillus subtilis* subsp. *spizizenii* (NR_024931), 99%	+	++	+	+	83 ± 4	54 ± 4
A	Mc2Re-9	*Bacillus subtilis* subsp. *subtilis* (NR_027552), 99%	+	+	++	–	61 ± 3	38 ± 2
A	Mc2Re-18	*Bacillus subtilis* subsp. *subtilis* (NR_027552), 99%	+	+	++	–	82 ± 2	50 ± 6
A	Mc2Re-21	*Bacillus subtilis* subsp. *subtilis* (NR_027552), 99%	–	+	++	–	66 ± 5	46 ± 3
A	Mc3Re-13	*Bacillus subtilis* subsp. *subtilis* (NR_027552), 98%	+	+	+	+	61 ± 3	43 ± 3
A	Mc5Re-2	*Bacillus subtilis* subsp. *spizizenii* (NR_024931), 100%	+	+	+	–	89 ± 3	59 ± 3

Table 2. Continued

			Antagonistic activity towards‡					
							Meloidogyne incognita¶	
ARDRA group*	Isolate number	Closest database match† (accession number), similarity (%)	*Verticillium dahliae*§	*Rhizoctonia solani*§	*Fusarium culmorum*§	*Ralstonia solanacearum*	Dead J2 after 48 h (%)‖	Mortality rate (% J2 per day)**
A	Mc5Re-15	*Bacillus subtilis* subsp. *subtilis* (NR_027552), 99%	+	+	+	+	33 ± 2	22 ± 1
A	Sd2Re-10	*Bacillus mojavensis* (NR_024693), 100%	++	++	++	–	52 ± 7	24 ± 2
A	Sd8Re-6	*Bacillus subtilis* subsp. *spizizenii* (NR_024931), 100%	+	+	+	+	22 ± 2	13 ± 2
A	Sd8Re-7	*Bacillus subtilis* subsp. *subtilis* (NR_027552), 99%	++	++	++	–	24 ± 2	12 ± 1
A	Sd8Re-23	*Bacillus subtilis* subsp. *spizizenii* (NR_024931), 100%	++	+	+	–	26 ± 2	14 ± 1
C	Wb1-13	*Bacillus endophyticus* (NR_025122), 99%	–	+	+	–	21 ± 2	14 ± 2
C	Mc4-18	*Bacillus endophyticus* (NR_025122), 99%	–	+	+	–	56 ± 5	21 ± 2
D	Wb2-3	*Paenibacillus polymyxa* (NR_037006), 99%	–	+	+	–	49 ± 4	34 ± 4
D	Sb3-1	*Paenibacillus kribbensis* (NR_025169), 99%	+++	++	+	–	44 ± 6	23 ± 1
D	Mc2-9	*Paenibacillus brasilensis* (NR_025106), 99%	++	++	+	–	64 ± 6	24 ± 1
D	Mc5-5	*Paenibacillus brasilensis* (NR_025106), 99%	++	–	++	–	58 ± 5	26 ± 1
D	Mc6-4	*Brevibacillus limnophilus* (NR_024822), 99%	+++	–	++	–	77 ± 4	39 ± 2
D	Mc2Re-16	*Paenibacillus brasilensis* (NR_025106), 98%	++	+	–	–	57 ± 9	31 ± 4
D	Mc5Re-14	*Paenibacillus polymyxa* (NR_037006), 99%	++	+	++	–	52 ± 3	38 ± 1
D	Sd5Re-24	*Paenibacillus brasilensis* (NR_025106), 99%	++	+	++	–	20 ± 2	11 ± 2
E	Wb1n-4	*Streptomyces scabiei* (NR_025865), 98%	+	++	+	+	70 ± 2	47 ± 4
E	Wb2n-2	*Streptomyces peucetius* (NR_024763), 98%	++	++	+	+	66 ± 3	40 ± 1
E	Wb2n-11	*Streptomyces subrutilus* (NR_026203), 99%	+++	+++	+	+	76 ± 7	48 ± 6
E	Wb2n-23	*Streptomyces peucetius* (NR_024763), 98%	++	+++	+	–	26 ± 3	15 ± 1
F	Mc1-3	*Lysobacter enzymogenes* (NR_036925), 99%	+	++	++	–	63 ± 6	23 ± 2

*The letters represent the different amplified rRNA gene restriction analysis patterns (A-F); group B (*Bacillus cereus* group) was completely excluded (Köberl *et al.*, 2011).

†According to 16S rRNA gene sequencing.

‡Dual culture assay: +…0–5 mm, ++…5–10 mm, +++…> 10 mm radius of zone of inhibition, –…no suppression.

§Results of a previous study performed by Köberl *et al.* (2011).

¶Control with *Escherichia coli* showed 28% dead J2 after 48 h, and a mortality rate of 21%, at controls with media and water both values were 0%.

‖± Standard deviation.

**Determined by linear regression of the percentages of dead J2 after 0, 6, 12 and 24 h, ± error of slope.

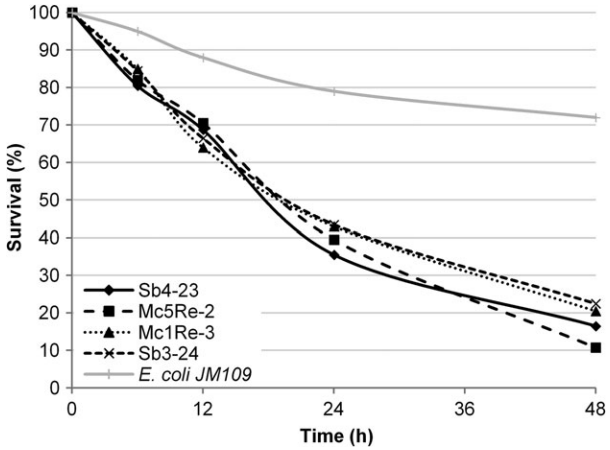

Fig. 3. *In vitro* effects of extracellular bacterial products on the mortality of *Meloidogyne incognita* juveniles. Depicted are the impacts of the four most efficient isolates in comparison with the control with *Escherichia coli* JM109.

Actinobacteria-specific probe, some of these bacterial colonies could be identified in the rhizosphere of *Matricaria chamomilla* as well when grown under organic desert farming conditions (Fig. 4).

Discussion

One of the major challenges of the 21st century will be to develop an environmentally sound and sustainable crop production. Desert agriculture opens up new possibilities to address diverse problems: to produce enough food for poor regions, to produce renewable crops for industrial applications, and to capture and restore CO_2 in soil. The accumulation of soilborne pathogens is another important ecological problem, which can cause dramatic yield losses. To solve this problem, we analysed associated microbial communities, which were found specific for each plant species and microhabitat. ITS profiles of fungal communities were less discriminative than bacterial fingerprints and characterised mainly by potential pathogens. Therefore, we selected bacterial antagonists against these and the well-known pathogens.

The dominance of Gram-positive bacteria in the group of antagonists in plant-associated and soil communities under arid conditions is in contrast to other studies performed under humid, temperate climate conditions. Here, mainly members of the genus *Pseudomonas* were found as antagonists (Berg *et al.*, 2006; Costa *et al.*, 2006; Weller, 2007), as it is well-studied for its beneficial plant–microorganism interaction (Haas & Défago, 2005; Lugtenberg & Kamilova, 2009). To verify our result, *Pseudomonas*-selective medium was used to monitor *Pseudomonas* isolates (King *et al.*, 1954), but only a few colonies were detected (data not shown). This differing ecology between arid and humid environments can be explained by the extreme abiotic conditions, such as the combination of extreme temperatures and desiccation, high soil salinity, low nutrient levels and high UV radiation levels in deserts. Recently, in a farm located in the northwestern desert region of Egypt, Marasco *et al.* (2012) reported a predominant role of *Bacillus* within the plant growth-promoting microbiome associated with the drought-sensitive pepper plant, which supported this conclusion. In addition, in the rhizosphere of Antarctic vascular plants, another extreme environment, *Firmicutes* were also identified as the most abundant phylum using a deep-sequencing approach (Teixeira *et al.*, 2010). However, in the microbiome of the sugar beet rhizosphere, *Firmicutes* represent 20% of the bacterial phyla with *Proteobacteria* as the dominant member (39%) (Mendes *et al.*, 2011). *Bacillus*, *Paenibacillus* and *Streptomyces* are spore-forming bacteria, and spore production aids in survival under suboptimal conditions

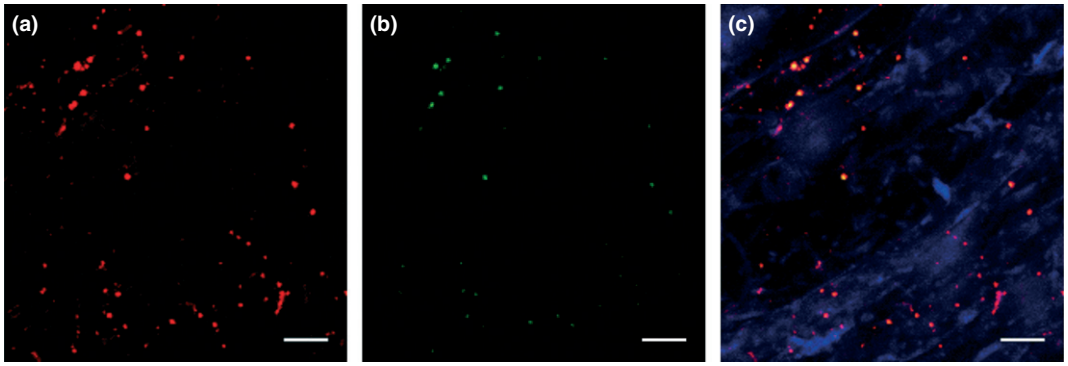

Fig. 4. *In situ* visualisation of *Actinobacteria* in the rhizosphere of *Matricaria chamomilla*. Fluorescent *in situ* hybridisation (FISH) showed a high colonisation of chamomile roots with bacteria in general (a), of which some colonies could be identified as *Actinobacteria* (b). The overlay (c) of the fluorochrome signals (a and b) with the autofluorescence of the root (blue) shows examples for *Actinobacteria* (yellow) amidst other eubacteria (red). Scale bar = 5 μm.

(Nicholson, 2002). However, it is still unclear whether these Gram-positive bacteria were alive and active in soil. Once considered their habitat, the soil may simply just serve as a reservoir (Hong *et al.*, 2009). While rhizosphere colonisation was recently shown by the BCA *Bacillus amyloliquefaciens* FZB42 (Fan *et al.*, 2011), we also found *Actinobacteria* colonisation as well.

Bacillus/*Paenibacillus* and *Streptomyces* species are well-known for their biocontrol potential (Schisler *et al.*, 2004; Berg, 2009). Several strains of *Bacillus subtilis* are already in use as biological pesticides (Fan *et al.*, 2011), and the antagonistic potential of *Paenibacillus polymyxa* towards a wide range of mycotoxin-producing fungi such as *F. culmorum* is well documented (Tupinambá *et al.*, 2008). Furthermore, a broad disease-suppressive activity has been detected for strains of *Lysobacter* (Postma *et al.*, 2011), the only Gram-negative genus selected. Despite this fact, we know that the biocontrol effect and mode of action are strongly strain-specific (Berg *et al.*, 2006; Berg, 2009). In our study, we detected plant species and microhabitat-specific bacterial antagonists, but also strain specificity was confirmed. Altogether, 13 broad-spectrum antagonists with antibacterial, antifungal and nematicidal activity were found which belong to seven different bacterial species of the genera *Bacillus* (*B. atrophaeus*, *B. mojavensis*, *B. subtilis* subsp. div., *B. vallismortis*) and *Streptomyces* (*S. peucetius*, *S. scabiei*, *S. subrutilus*). On their basis, biocontrol products specifically for arid conditions can be developed.

In this study, we linked ecological data with the selection strategy for antagonists. Within the fungal community, mainly potential phytopathogens were identified. Therefore, we focused on the selection of bacterial antagonists. In the cultivation-independent and dependent approach, strains of *Bacillus*/*Paenibacillus* were found as the key players in bacterial communities in arid agricultural systems. Conversely, members of the genus *Streptomyces* were important in the natural desert ecosystem. This was also confirmed by a comparative deep-sequencing approach of desert and field soil (Köberl *et al.*, 2011). Gram-positive, spore-forming bacteria of the genera *Bacillus*, *Paenibacillus* and *Streptomyces* were selected using our hierarchical procedure; all of them belong to risk group 1 (no risk for humans and the environment) and are promising drought-resistant and heat-resistant biocontrol candidates. Furthermore, they showed a remarkable antibiotic activity.

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