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Research Article

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RESEARCH ARTICLE

Endophytic bacterial communities of oilseed rape associate with genotype-specific resistance against *Verticillium longisporum*

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One sentence summary: Endophytic bacterial communities of oilseed rape associate with genotype-specific resistance against *Verticillium longisporum*.

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ABSTRACT

Associations of endophytic bacterial community composition of oilseed rape (*Brassica napus* L.) with quantitative resistance against the soil-borne fungal pathogen *Verticillium longisporum* was assessed by 16S rRNA gene amplicon sequencing in roots and hypocotyls of four plant lines with contrasting genetic composition in regard to quantitative resistance reactions. The plant compartment was found to be the dominating driving factor for the specificity of bacterial communities in healthy plants. Furthermore, *V. longisporum* infection triggered a stabilization of phylogenetic group abundance in replicated samples suggesting a host genotype-specific selection. Genotype-specific associations with bacterial phylogenetic group abundance were identified by comparison of plant genotype groups (resistant versus susceptible) and treatment groups (healthy versus *V. longisporum*-infected) allowing dissection into constitutive and induced directional association patterns. Relative abundance of *Flavobacteria*, *Pseudomonas*, *Rhizobium* and *Cellvibrio* was associated with resistance/susceptibility. Relative abundance of *Flavobacteria* and *Cellvibrio* was increased in resistant genotypes according to their known ecological functions. In contrast, a higher relative abundance of *Pseudomonas* and *Rhizobium*, which are known to harbor many species with antagonistic properties to fungal pathogens, was found to be associated with susceptibility, indicating that these groups do not play a major role in genetically controlled resistance of oilseed rape against *V. longisporum*.

Keywords: *Verticillium longisporum*; disease resistance; *Brassica napus*; endobiome; 16S rRNA gene amplicon Illumina sequencing

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INTRODUCTION

Plant-associated microbiota including bacterial and fungal organisms are key determinants for plant growth, health and yield (Müller *et al.* 2016). Their genomes, sometimes referred to as the 'second genome of plants', can be considered a fundamental part of the plant holobiome (Berg *et al.* 2014; Vandenkoornhuyse *et al.* 2015). Ectophytic and endophytic bacteria and fungi can suppress or prevent successful plant colonization by fungal and bacterial soil-borne pathogens. Both, rhizosphere ectophytic bacteria as well as internal root endophytic bacteria have been shown to indirectly trigger systemic plant defense responses, to confer broad-spectrum resistance and to directly antagonize soil-borne pathogen development (van Wees *et al.* 2008; Berendsen, Pieterse and Bakker 2012; Santoyo *et al.* 2016; Durán *et al.* 2018). It has been observed that plants harboring highly diverse bacterial communities in the rhizosphere and roots are often more resistant to fungal pathogen invasions compared with plants harboring only bacterial communities with limited diversity. However, the mode of action and underlying mechanisms of health promotion seems to be complex and is poorly understood (Wei *et al.* 2015).

It has been shown for a number of plant species including oilseed rape, that the rhizosphere microbial communities are strongly affected by the location, soil type and developmental stage (e.g. Berg *et al.* 2005; Berg and Smalla 2009; Schreiter *et al.* 2014). In contrast, the influence of the plant genotype or cultivar in crop plants on the diversity of rhizosphere and root endophytic microbial communities has been less intensively investigated. In *Arabidopsis thaliana*, a close relative of *Brassica napus* (oilseed rape), the plant genotype was found to substantially affecting the microbiota diversity (Bulgarelli *et al.* 2012; Lundberg *et al.* 2012). Only a few studies have been carried out in crop plants, for example, it has been documented that the root bacterial endophytic community of modern wheat cultivars is more diverse than that seen for ancient wheat land races and older cultivars. In addition, certain bacteria species were selected for by the plant genotype within the roots of modern wheat cultivars suggesting that these bacteria might be exploited by specific plant genotypes as plant growth-promoting bacteria (Germida and Siciliano 2001).

Infection of plants with soil-borne pathogens leads to a shift in bacterial community composition in the rhizosphere (Zhang *et al.* 2011; Berendsen, Pieterse and Bakker 2012). Factors shaping the composition of the root bacterial endophytic communities are not as well investigated as for the rhizosphere. However, it seems that in Arabidopsis soil bacteria are strongly selected by the host species at the root surface for entry into the root interior (Durán *et al.* 2018). The strong selection of bacteria endophytes at the root surface suggests that a plant-based genetic control mechanism of this process exists which might result in plant genetic variation linked to genotype-specific disease resistance against soil-borne fungal pathogens. However, to our knowledge genotype- and/or resistance/susceptibility-specific association of endophyte bacteria communities have not yet been assessed.

Verticillium longisporum is a soil-borne fungal pathogen of oilseed rape which enters the plant through wounds, or by direct penetration into epidermal cells of lateral roots or root hairs (Zhou *et al.* 2006). *Verticillium longisporum* hyphae then enter the xylem, spread inside the plants from the root through the hypocotyl tissue and cause premature ripening of oilseed rape known as *Verticillium* stem striping disease (Depotter *et al.* 2016). Nothing is known about the role of endophytic bacterial communities associated with root and hypocotyl tissues in the rapeseed-*V. longisporum* pathosystem and about their association with *V. longisporum* resistance.

Quantitative partial resistance to *V. longisporum* infection has been documented in oilseed rape. However, successful invasion of roots has been described both for partially resistant as well as for highly susceptible genotypes (Eynck *et al.* 2009). Quantitative resistance seems to be expressed internally within the xylem of the hypocotyl tissue and is associated with deposition of phenolic compounds (Obermeier *et al.* 2013). It was not considered so far if specific bacterial communities present in the endophytic compartment of the roots and the hypocotyl of the partially resistant genotypes contribute to the resistance expression.

In this study, we compared the community profiles of endophytic bacteria colonizing roots and hypocotyls of two susceptible and two partially resistant rapeseed genotypes using a 16S rRNA gene amplicon-sequencing based approach to gain a first insight into the contribution of the diversity and community composition of the bacterial root microbiota on the plant genotype-specific resistance against *V. longisporum*. The following questions were addressed: (i) Do genotype-specific endophytic bacterial communities occur in roots and hypocotyls? (ii) If differences occur, are these mainly linked to differences in the diversity or the phylogenetic composition of the bacteria communities? (iii) Does the presence of *V. longisporum* affect the endophytic root and/or hypocotyl bacterial communities? (iv) Can specific bacterial community patterns be correlated to susceptibility or resistance of the plant genotypes? In addition, we aimed to identify potential key bacterial phylogenetic groups which could be linked to the root and hypocotyl plant compartments of the resistant genotypes.

MATERIALS AND METHODS

Rapeseed lines

Four different winter oilseed rape doubled haploid (DH) lines were investigated, DH12-R and DH106-R (partially resistant to *V. longisporum*) and DH24-S and DH147-S (susceptible to *V. longisporum*). The four DH lines were produced from a cross between a moderately *V. longisporum*-resistant line Express617 and a strongly resistant resynthesized line R53 (produced from a kale, *B. oleracea* var. *acephala*, crossed with a chinese cabbage, *B. rapa* var. *pekinensis*) (Obermeier *et al.* 2013).

Fungal inoculum

Stock culture of *V. longisporum* isolate VL43, originally isolated from *Brassica napus* (Zeise and von Tiedemann 2002), was grown in potato dextrose broth medium for 10–14 days at 23°C and 120 rpm. The culture was filtered using a sterile fine cloth to remove the fungal mycelia. The concentration of spores in the resulting suspension was measured using a Fuchs-Rosenthal counting chamber and adjusted to 1×10^6 spores mL^{-1} with potato dextrose broth medium for inoculation of seedlings.

Greenhouse experiment

The soil used in the experiments was obtained from a field station in Groß Gerau, Hesse, Germany (49.940022, 8.503699

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Decimal Degrees, sandy fluvisol) where the top soil (upper 30 cm) was collected from a field where no oilseed rape was grown before. The bulk soil was sieved and mixed with autoclaved sand (1:1) and used for growing the plants in the greenhouse. Seeds were surface sterilized with 70% ethanol for 30 min and washed three times with autoclaved pure water. Hundred plants (four contrasting genotypes, 25 plants per genotype, scored at three time points) were grown in the soil-sand mixture in a greenhouse with a day-night cycle of 16 hours of light (light intensity: artificial lightning 10 000 lux) at 22°C and 8 hours of dark at about 18°C. About 14 days after sowing, seedlings were carefully removed from the soil-sand mixture and the roots were gently washed under tap water. About 2 cm of the root tips were cut by a sterile scissor and 34 of the seedlings for each genotype were directly transferred to autoclaved pure water for 30 min as mock control (non-inoculated control). A total of 66 of the seedlings for each genotype were used for root dip-inoculation by *V. longisporum* with a concentration of 1×10^6 spores mL^{-1} prepared as describe above for 30 min (Eynck *et al.* 2009), on a pre-sterilized working bench. After inoculation, the seedlings were transferred to new containers and grown in greenhouse under the same standardized conditions as described above for up to 4 weeks (28 days after inoculation, dai). On day 41 the plants were harvested as described by Bulgarelli *et al.* (2012) with slight modifications. Roots and hypocotyls were separated by using a sterile scissor and transferred to two different sterile 50 mL tube containing PBSS buffer. The tubes were horizontally shaken for 20 min at 180 rpm and transferred to a new tube with PBSS buffer where the procedure was repeated. Plant parts were washed in autoclaved pure water, transferred to a new tube with PBSS buffer and sonicated for 5 min. After removing the washing buffer the plant parts were frozen at −20°C until DNA extraction.

Disease scoring

For disease scoring for each genotype a total of 10 *V. longisporum*-inoculated and 10 mock-inoculated plants were scored weekly from 7 to 28 days after inoculation (dai). The assessment based on a scoring scale from one (no symptoms) to nine (plant is dead) as described by Eynck *et al.* (2009). The mean disease severity area under the disease progress curve (mean AUDPC) values was calculated according to Eynck *et al.* (2009). The netAUDPC values were calculated by subtracting the values of the mean AUDPC for the mock- and *V. longisporum*-inoculated data set.

Total DNA extraction from plant material

Hypocotyl and root samples of the four different genotypes and the two different treatments (mock, *Verticillium*-infected) were analyzed in triplicates (samples derived from three individual pots), resulting in a total number of 48 samples. First, the plant tissue was ground in liquid nitrogen to a fine powder using autoclaved and UVC (ultra violet light lambda = 254 nm) exposed mortars and pestles. DNA extraction was performed with the NucleoSpin soil DNA extraction kit of Macherey Nagel (Weilmünster, Germany) according to manufacturer's instructions. The DNA concentration was determined using a NanoDrop 2000c spectrophotometer (Thermo Scientific) and adjusted to DNA concentrations of 15 ng μL^{-1} (for qPCR) and 10 ng μL^{-1} (for Illumina MiSeq analysis).

Quantification of *V. longisporum* in root and hypocotyl samples via qPCR

Roots and hypocotyl samples were harvested from a total of 15 individual plants per genotype (five individuals per pot) at 28 dai. All samples were rinsed and stored at −20°C until further use. As positive control for qPCR analysis *V. longisporum* isolate VL43 was grown as described above and fungal mycelium was isolated by filtration and lyophilized for one day. Total plant-derived DNA and fungal genomic DNA were extracted using a CTAB protocol (Doyle and Doyle 1990) and DNA was quantified using the Qubit 2.0 fluorometer (Thermo Fisher Scientific, Germany). A ViiA7 Real-Time PCR System (Thermo Fisher Scientific, Germany) was used for qPCR analysis. A 261 bp fragment of the fungal ribosomal rRNA gene operon internal transcript spacer (ITS) region of *V. longisporum* was amplified using the primer pair OLG70/OLG71 (Knüffer *et al.* 2017). A qPCR reaction of 10 μL total volume contained 15 ng DNA, 0.3 μM of each primer and 1x FastStart Universal SYBR Green I Master (including Rox) (Roche, Mannheim, Germany). A *V. longisporum* standard curve in a range of 1 ng to 0.01 pg fungal genomic DNA of the isolate VL43 was used. For each DNA sample three technical replicates were measured and mean values were calculated. The following PCR program was used: 95°C for 15 min (enzyme activation), 40 cycles of 95°C for 10 s, 60°C for 15 s and 72°C for 15 s and finally 72°C for 2 min. Subsequently melting curve analysis was performed for evaluation of the amplification specificity.

Bacterial community analysis by 16S rRNA gene amplicon Illumina MiSeq sequencing

Bacterial communities were analyzed by 16S rRNA gene amplicon based high-throughput sequencing (next-generation sequencing (NGS)) using the bacterial 16S rRNA gene targeting primers 341F (5´-CCTACGGGNGGCWGCAG-3´) and 785R (5´-GACTACHVGGGTATCTAAKCC-3´) flanking the V3-V4 regions (Klindworth *et al.* 2013). PCR amplification, tagging, equimolar mixing of the 48 samples, clean-up and Illumina sequencing with the 300 bp paired-end read Illumina MiSeq V3 chemistry were performed by LGC Genomics (Berlin, Germany). Demultiplexing of the sequence libraries was performed with the Illumina bcl2fastq 1.8.4 software, reads were sorted by amplicon inline barcodes. Reads with missing barcodes, one-sided barcodes or conflicting barcode pairs were discarded. Barcodes, adaptors and primer sequences were clipped after sorting. Reads with a final length < 100 bp were discarded after adaptor clipping. All the Illumina processed reads for 48 sequenced samples have been deposited at NCBI for project PRJNA586899 and are available under BioSample accession numbers SAMN13174421 to SAMN13174468. Sequences were oriented into the forward and reverse primer direction. Forward and reverse reads were combined using BBMerge 34.48 (http://bbmap.sourceforge.net/). Data sets of combined reads were analyzed in the SILVAngs analysis pipeline (https://www.arb-silva.de/ngs/). In brief, 16S rRNA gene amplicon reads were aligned with the SILVA Incremental Aligner (SINA v 1.2.10 for ARB SVN (revision 21 008)) (Pruesse, Peplies and Glöckner 2012) against the SILVA SSU rRNA SEED and quality controlled (Quast *et al.* 2013). For quality control, the following reads were identified and excluded from further processing: reads shorter than 50 aligned nucleotides, reads with more than 2% of ambiguities or 2% homopolymers, reads with putative contaminations and artefacts that were seen as reads with a low alignment quality (50 alignment identity, 40 alignment score reported by SINA). After quality control,

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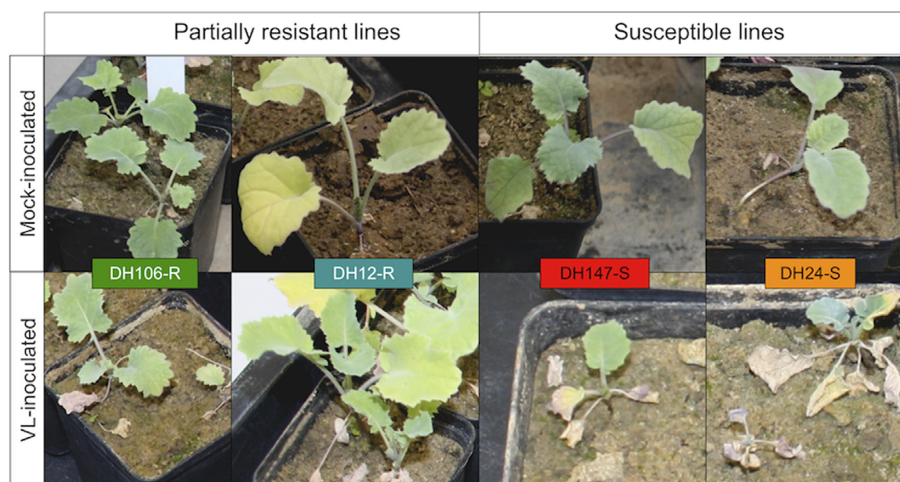

Figure 1. Symptoms of mock- (above) and *V. longisporum*-inoculated (below) rapeseed plants 28 days after inoculation for partially resistant lines (DH106-R, DH12-R) and susceptible lines (DH147-S, DH24-S).

identical reads were identified (dereplication), unique reads were clustered (operational taxonomic units, OTUs) and the reference read of each OTU was classified. Dereplication and clustering was processed with cd-hit-est (version 3.1.2; http://www.bioinformatics.org/cd-hit) (Li and Godzik 2006) using the accurate mode. Overhangs were ignored and identity criteria of 1.00 and 0.98, respectively were applied. The classification was performed by BLAST search against the non-redundant version of SILVA SSU Ref data set (release 128; http://www.arb-silva.de) using blastn (version 2.2.30+; http://blast.ncbi.nlm.nih.gov/Blast.cgi) with standard settings (Camacho *et al.* 2009). The classification of each OTU read was mapped onto all identical reads belonging to the respective OTU, resulting in quantitative information (number of individual reads per taxonomic path) within the limitations of PCR and sequencing technique biases, as well as, multiple rRNA operons. Reads without BLAST hits or reads, where the function '(% sequence identity + % alignment coverage)/2" did not exceed the value of 93, remain unclassified. These reads were assigned to the meta group 'No Relative' in the SILVAngs fingerprint and Krona charts (Ondov, Bergman and Phillippy 2011). This approach was first used by Ionescu *et al.* (2012) and Klindworth *et al.* (2013). Statistical analysis at the level of bacterial phyla and phylogenetic group data was conducted with PAST3 software version 3.11 (Hammer, Harper and Ryan 2001). Non-metric multidimensional scaling (nMDS) and hierarchical clustering based on a distance or dissimilarity matrix generated with the Bray–Curtis dissimilarity index was used to obtain the pairwise dissimilarity between bacterial community compositions among individual samples. Statistical significant differences among samples was on a global scale determined by ANOSIM analysis performed in PAST3 and for pairwise comparisons by using PERMANOWA analysis performed in Primer-e. Principal component analysis (PCA) including biplot data based on a co-variance matrix was used to determine main phyla or phylogenetic groups which contributed to the differences among samples. Similarity percentages breakdown (SIMPER) analysis based on the distance/similarity measurement of the bacterial community profiles using the Bray–Curtis dissimilarity index was used to identify the major contributors to differences between groups. Figures showing diversity index values and analysis of variance (ANOVA) from data obtained by PAST3 were created with Sigma Plot software version 13. Alpha-diversity analysis included determination of the Chao 1 index, which gives information about the richness using the total number of species per sample, the Shannon index, which shows the proportion of individuals of one phylogenetic group to the total number of individuals and dominance, which shows if the community is dominated by single phylogenetic groups.

RESULTS

Development of *V. longisporum* disease and infection in contrasting oilseed rape lines

Well characterized oilseed rape lines were selected which harbor a different genetic composition for two major quantitative trait loci (QTL) for *V. longisporum* resistance but share a similar composition of the background genome. This was achieved by their selection from a total of 214 DH offspring from a cross of a partially resistant (Express617) and a strongly resistant (R53) parental oilseed rape line. Two sets of strongly contrasting DH lines (highly susceptible and partially resistant) were selected based on (i) resistance/susceptibility allele composition for two SSR markers each flanking two major *V. longisporum* resistance QTL, on chromosomes C1 and C5, based on (ii) contrasting reproducible greenhouse phenotyping results (low standard deviation) from two independent greenhouse screening experiments and based on (iii) low pairwise genetic background genome differences (Nei's distance) using 207 SSR markers (Obermeier *et al.* 2013). Diseases symptoms typical induced by *V. longisporum* infection of young seedlings including yellowing of cotyledons and older leaves and black veins were observed 14 days after inoculation (dai) in the susceptible lines DH24-S and DH147-S and only to a limited extend in the partially resistant lines DH12-R and DH106-R. A clear visual differentiation between susceptible and partially resistant lines was possible at 28 dai (Fig. 1). The disease progress expressed as netAUDPC (net area under the disease progress curve) values permitted the grouping of the four lines based on their quantitative resistance level (Table 1 and Fig. 2). Low scored netAUDPC values of 4.9 and 7.7 for lines DH12-R and DH106-R indicated their partial resistance. Significantly higher netAUDPC values of 17.15 and 24.15 were recorded for line DH24-S and DH147-S, respectively, indicating susceptibility against *V. longisporum* infection. The quantification of *V. longisporum* 28 dai in three individual plant roots and hypocotyls of each lines by qPCR confirmed the infection of the inoculated

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Table 1. Mean disease scoring for four *Brassica napus* doubled haploid (DH) lines inoculated with *Verticillium longisporum*.

DH lines	DH12-R		DH24-S		DH106-R		DH147-S	
Time point (dai)	mock	VL	mock	VL	mock	VL	mock	VL
7	1 (±0.00)a	1 (±0.00)a	1 (±0.00)a	1 (±0.00)a	1 (±0.00)a	1 (±0.00)a	1 (±0.00)a	1 (±0.00)a
14	1 (±0.00)a	1.2 (±0.42)a	1.2 (±0.42)a	1.4 (±0.69)b	1.2 (±0.42)a	1.3 (±0.48)b	1 (±0.00)a	1.6 (±0.84)b
21	1.1 (±0.31)a	1.3 (±0.48)b	1.4 (±0.96)a	2.2 (±1.61)a	1.3 (±0.48)a	1.7 (±1.05)b	1 (±0.00)a	1.8 (±1.13)a
28	1.1 (±0.31)a	1.3 (±0.48)a	1.4 (±0.96)a	3.3 (±1.82)b	1.3 (±0.48)a	2 (±1.05)a	1 (±0.00)a	3.7 (±2.58)b
AUDPC	32.9	37.8	39.2	56.3	37.8	45.5	31.5	55.6
netAUDPC	4.9 (±6.7)		17.1 (±12.6)		7.7 (±8.61)		24.15 (±29.7)	

Standard deviation is shown in brackets. Statistical comparisons have been performed separately for each DH line and time point. Alphabetic annotation shows significant differences at $P < 0.05$ (least significant difference).

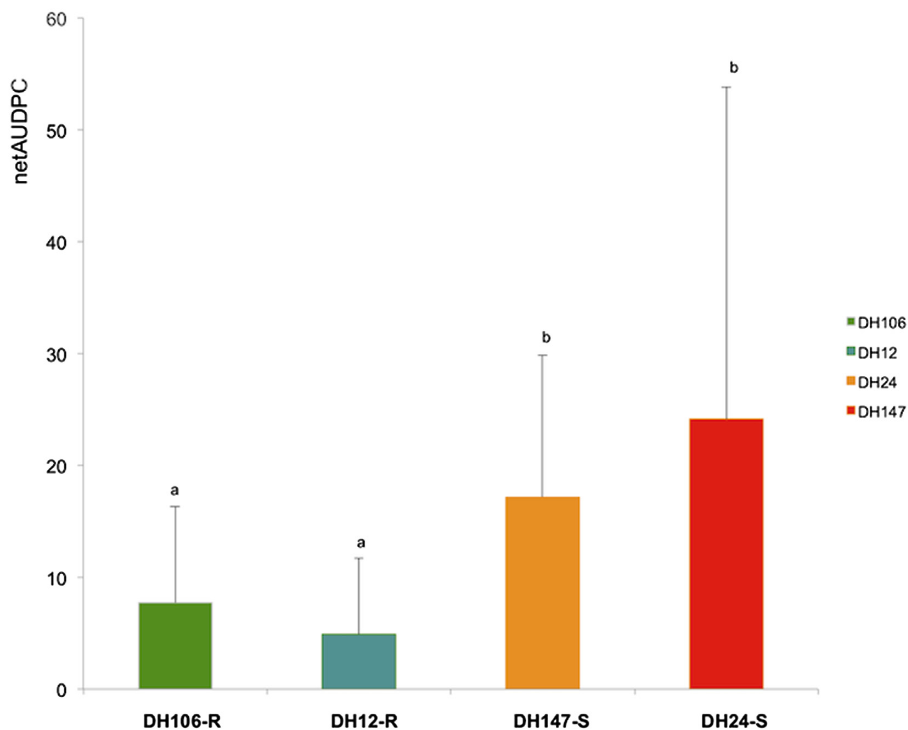

Figure 2. Area Under the Disease Progress Curve (netAUDPC) for four different lines and standard deviation from n = 10 individual plants. The two lines on the right are susceptible lines, the two lines on the left are partially resistant lines. Alphabetic annotations show significant differences based on pairwise comparison for each cultivar (LSD post hoc test, $P < 0.05$).

plants while *V. longisporum* was not detected in mock-inoculated control plants (Fig. 3). The average amount of *V. longisporum* DNA detected in roots of inoculated plants was in the same range for inoculated plants of all four lines, ranging from 0.1 to 0.3 ng *V. longisporum* DNA per 15 ng total genomic DNA. In contrast, the average amount of *V. longisporum* DNA in hypocotyl samples of inoculated plants was substantially higher in the susceptible compared to the partially resistant lines.

Overview of 16S rRNA gene amplicon sequencing data generated from root and hypocotyl-associated bacterial communities

A total of 2382 776 combined high-quality paired-end 16S rRNA gene sequence reads were obtained from the DNA samples extracted from 48 samples (4 lines × 3 biological replicates × 2 treatments: infected/non-infected x 2 tissues: hypocotyl/roots). Only 3025 reads were rejected after quality control and excluded from analysis. The average length of the combined reads was 406 nt. Alignment with SILVA SSU rRNA SEED database and OTU clustering revealed that a fraction of 1569,734 (64.4%) and 440,622 (17.8%) of these quality-filtered reads represented host chloroplast and mitochondrial sequences, respectively (Fig. S1, Supporting Information). Only 19 reads were assigned to the domain *Archaea* confirming the specificity of the used primer pairs for the domain *Bacteria* including endosymbontic *Bacteria*-derived chloroplast and mitochondria organelles (Thijs *et al.* 2017). Only 1285 reads could not be classified. The relative abundance of reads representing non-*Bacteria* was lower in sequence data sets from roots (76.3%) compared to hypocotyl (88.4%). Sequences, representing chloroplasts, mitochondria, and *Archaea*, along with reads that could not be classified (no relative) were excluded from further analyses and *Bacteria* derived 16S rRNA gene sequences were set as 100%. After final filtering, a total of 372 420 high-quality reads representing

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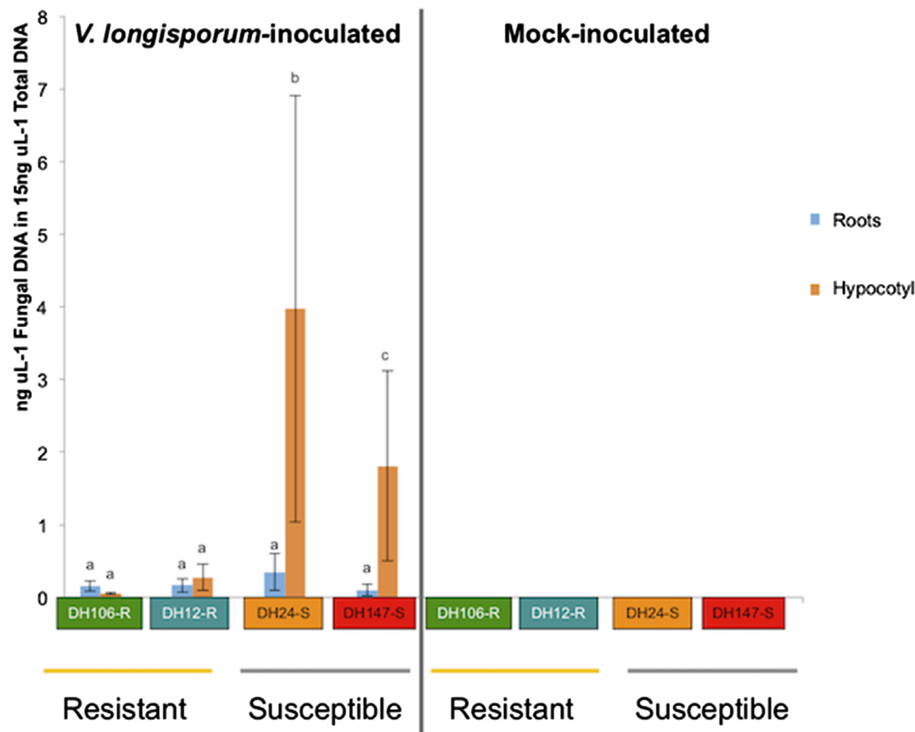

Figure 3. Comparison of fungal DNA measured by qPCR in hypocotyls at 28 days after inoculation for four susceptible and partially resistant DH lines (thin lines indicate standard deviation of three individual plants). Alphabetic annotations show significant differences based on pairwise comparison for each cultivar (LSD post hoc test, $P < 0.05$).

Bacteria were retained (on average 7669 per sample) (Fig. S1 and Table S1, Supporting Information). Clustering of those *Bacteria* reads resulted in 238 078 OTUs using a 98% sequence similarity cutoff value. The variation in the number of reads detecting bacterial phylogenetic groups between biological replicates was shown to have no impact on the differences in diversity composition analyzes *Bacteria*-assigned sequences and respective OTUs were classified into 1031 phylogenetic groups (mostly representing specific genera) within 36 phyla. The phylogenetic groups were mainly assigned at the genus level or higher taxonomic levels or labeled as uncultured group if a genus level assignment was not possible. Subsequent references to microbiota thereby focus only on the fraction of *Bacteria* (bacterial community); fungal and archaeal members of the total microbiota were not considered in the following analyzes.

Effects of plant compartment and genotype on the bacterial community patterns in healthy rapeseed

First, hypocotyl and root endophytic bacterial communities were compared for mock-inoculated plants to elucidate the differences among the bacterial communities present in those compartments and the variation among the rapeseed lines. Nonmetric multidimensional scaling (nMDS) ordination of the bacterial community patterns resolved at the level of phylogenetic groups compared based on Bray–Curtis distance matrices showed a clear separation of bacterial communities present in the hypocotyl and roots. Endophytic communities of the same compartment (root or hypocotyl) were similar for the different rapeseed lines (Fig. 4A), except, the root endophytic communities of line DH24 did not show an overlap with the root endophytic communities of the other three rapeseed lines in the nMDS plot. The plant compartment was therefore the dominating driving factor for the specificity of hypocotyl and root endophytic *Bacteria* communities. Box-plot analysis based on Bray–Curtis similarities of the bacterial community profiles indicated that the variability among the bacterial community patterns of the biological replicates was much more stable among individual plants for the root than for the hypocotyl endophytic bacterial communities (Fig. 4B).

The diversity of the bacterial communities was analyzed by comparison of the total number of phylogenetic groups (Chao index), by comparison of the distribution of the phylogenetic groups within the communities by the determination of dominance values and by comparison of the overall diversity of the communities using the Shannon index which considered both factors. The number of phylogenetic groups (chao index) was, for both compartments, in the same range with a Chao1 index of 384.6–463.8 for hypocotyl and 181.8–626.3 for root endophytic bacterial communities (Fig. 4C). The diversity indices of the root endophytic communities were slightly higher and the dominance of individual phylogenetic groups within the root endophytic communities slightly lower. The comparison of the diversity of root and hypocotyl endophytic bacterial communities showed that root-associated endophytic communities of all four rapeseed lines were characterized by a lower intra-replicate variation than hypocotyl-associated endophytic communities. In summary, all different types of analysis indicate that in healthy plants root communities were more similar between biological replicates, meaning more specific and stable, than hypocotyl communities. Distinct plant genotype-specific differences in the diversity of rapeseed root and hypocotyl bacterial communities were not observed.

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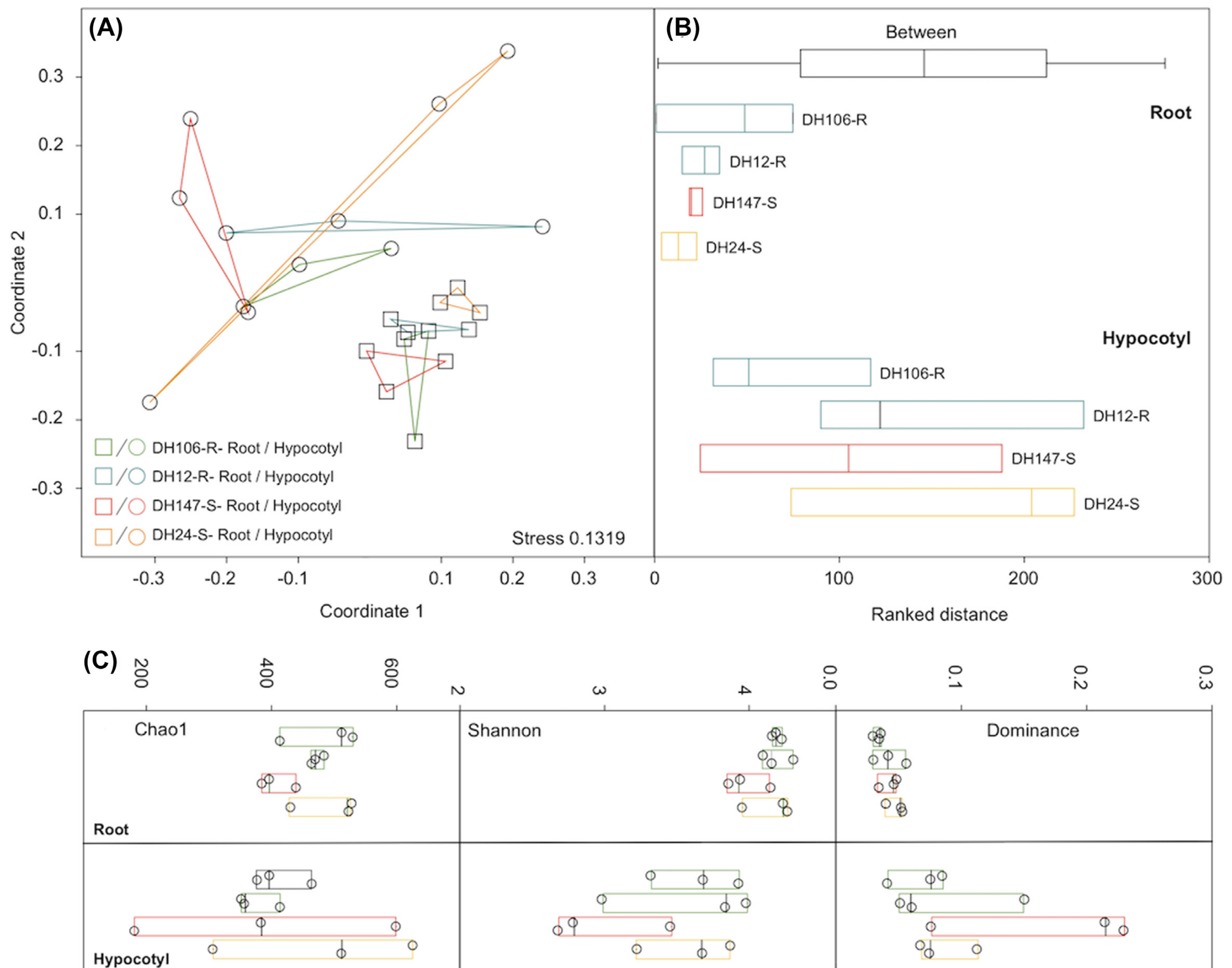

Figure 4. Phylogenetic composition of bacterial communities in roots and hypocotyls of mock-treated partially resistant rapeseed lines (DH12-R, DH106-R) and susceptible rapeseed lines (DH24-S, DH147-S). **(A)**, Non-metric multidimensional scaling (nMDS) analysis of relative abundance pattern of bacterial communities based on the phylogenetic group assignment. **(B)**, box plots of ranked distances analyzed at the level of phylogenetic groups. **(C)**, box plots of alpha diversity indices Chao 1, Shannon and Dominance index. Analysis (A) and (B) were performed in PAST3 and based on a Bray–Curtis similarity matrix calculation. Box plots were calculated with the interpolated quartile method. Diversity indices (C) were calculated in PAST3.

Phylogenetic composition and diversity of endophytic bacterial communities of root and hypocotyl bacterial communities in healthy rapeseed lines

Endophytic bacterial communities of both compartments (root, r and hypocotyl, h) were dominated by *Proteobacteria* (r: 58.7%–87.3%; h: 37.6–84%), *Bacteriodetes* (r: 5.9%–24.4%; h: 6.8%–50.5%), *Actinobacteria* (r: 1.8%–11.2%; h: 3%–32.2%), and *Verrucomicrobia* (r: 2.4%–6.7%; h: 0.4%–4.7%) which together accounting for > 87% of the reads obtained per sample (Fig. 4). PCA and SIMPER analysis were applied to determine phyla which had the main contribution to the differences between *Bacteria* communities colonizing the hypocotyl or the roots (Figs S2–S4, Supporting Information). PCA based on relative abundance patterns of phyla showed that *Proteobacteria* and *Bacteroidetes* had with 34% (each) the highest contribution to the differences between the roots and hypocotyls microbiota (Fig. S4, Supporting Information). Besides, *Actinobacteria* (14%) and *Verrucomicrobia*, *Planctomycetes* and *Saccharibacteria* (to a lower extend) also contributed to the compartment specificity of the endophytic bacterial communities. PCA plots showed no distinct separation between root and hypocotyl samples. Hypocotyl samples were widespread in PCA plot and differences were therefore driven by various groups (Fig. S4, Supporting Information).

A total of 42 phylogenetic groups occurred in root or hypocotyl bacterial communities of mock-inoculated plants in at least one of the four rapeseed lines with a relative abundance of 1% and above. The genera with the highest relative abundance in both compartments were *Flavobacterium* (*Bacteroidetes*) and *Rhizobium* (*Alphaproteobacteria*) (r: 9.2%–15.5%; h: 6.4%–36.2%). A total of 18 and 11 further phylogenetic groups occur with a relative abundance between 2% to 8% and 2% to 10% relative abundance in root and hypocotyl samples of at least one rapeseed line. Among those, eight further genera which were shared in a relative high abundance (>2%) by both compartments, *Caulobacter* (*Alphaproteobacteria*), *Acidovorax*, *Paucibacter*, *Methylotenea*, *Methylophilus* and *Massilia* (*Betaproteobacteria*) and *Pseudomonas* and *Cellvibrio* (*Gammaproteobacteria*). The further 10 genera specifically abundant root endophytes were *Streptomyces* (*Actinobacteria*), *Sphingomonas*, *Sphingopyxis*, *Shinella* and *Devosia* (*Alphaproteobacteria*), *Pelomonas*, uncultured *Comamonadaceae* and *Dechloromonas* (*Betaproteobacteria*),

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Pseudoxanthmonas (*Gammaproteobacteria*) and a phylogenetic group of uncultured *Verrucomicrobia*. The four phylogenetic groups specifically abundant in hypocotyl samples were the genera *Microbacterium* and *Leucobacter* (*Actinobacteria*), *Brevunimonas* (*Alphaproteobacteria*) and a phylogenetic group of uncultured *Saccharibacteria*. PCA and SIMPER analysis performed at the level of phylogenetic groups indicated that *Flavobacteria* (T1) had the strongest contribution to the differences between the roots and hypocotyl associated bacterial communities (12.9% with significant differences; T-test, $P > 0.05$). Next contributing genera were *Pseudomonas* (T3) (5.8%), *Rhizobium* (T2) (4.9% with significant differences) and *Cellvibrio* (T4) (4.1% with important differences) (Fig. S3, Supporting Information).

Shifts in the endophytic bacterial community composition and diversity after *V. longisporum* infection

Changes in the community composition of the root and hypocotyl bacterial endophytes were studied 28 dai of the four rapeseed lines with *V. longisporum* when infection had been confirmed by qPCR (see above). The infection triggered an increase of the total abundance of *Bacteria* in roots and hypocotyls (13.9%). Especially in susceptible genotypes, there was a high increase in roots of DH147 (50.5%) and in hypocotyls of DH24 (42.8%). However, the patterns of the root endophytic bacterial communities of the partially resistant rapeseed lines DH106-R and DH12-R did overlap in NMDs plots and were not altered after *V. longisporum* infection (Fig. 5A, green and blue lines), but the variation among biological replicates was lower among infected compared to mock-treated plants (controls). This indicated a higher stability of the endophytic bacterial community in resistant rapeseed lines triggered by *V. longisporum* infection (Fig. 5B). In contrast, bacterial community patterns of infected and mock-treated plants did not overlap in NMDs plots for the susceptible genotypes DH147-S and DH-24S (Fig. 5A, red and orange lines) indicating that the community patterns of the bacterial root endophytes of the susceptible rapeseed lines changed strongly in the presence of *V. longisporum*. Also, in contrast to the community patterns in the partially resistant rapeseed lines, the variability among the biological replicates for the susceptible lines in the roots increased after *V. longisporum* infection (Fig. 5B). A similar pattern was found for the endophyte bacteria community patterns in the hypocotyls. In the hypocotyl, the community patterns in the partial resistant rapeseed lines were not affected by *V. longisporum* infection indicated by the overlap of infected and control data sets in the NMDs plot (Fig. 5C). However, the variation among biological replicates for the partially resistant lines was strongly reduced after *V. longisporum* infections (Fig. 5D). In contrast, the composition of the hypocotyl bacterial communities of the susceptible rapeseed lines was different among mock-treated control and infected plants. Especially for line DH24-S community patterns of mock-treated and infected plants did not show any overlap in the NMDS plot. In contrast to the partially resistant rapeseed lines the variability among the community patterns of biological replicates only slightly deceased (line DH147-S) or were not affected (line DH24-S).

The diversity of the root and hypocotyl endophytic bacterial communities was not clearly altered after *V. longisporum* infection. The richness (Chao-index), the overall diversity (Shannon index), and the distribution of individual phylogenetic groups (dominance values) of the root-associated bacterial communities did not show clear differences, which were associated with *V. longisporum* infection (Fig. 5E). Only the partially resistant rapeseed lines showed bacterial communities with a high stability with respect to the community diversity (Shannon index) and a low dominance of individual phylogenetic groups irrespective of *V. longisporum* infection. The variation of the number of phylogenetic groups (Chao index), the community diversity (Shannon index) and the abundance of dominant phylogenetic groups among biological replicates increased especially for the root-associated bacterial communities for the susceptible line DH147-S after *V. longisporum* infection (Fig. 5E). The diversity of the endophytic communities of the hypocotyl was similar with respect to community richness (Chao index) compared to the root microbiota, but characterized by a slightly lower diversity with a higher abundance of few individual phylogenetic groups (higher dominance values) (Fig. 5F). For the partially resistant rapeseed lines the presence of *V. longisporum* did not change the overall diversity of the endophytic communities, but led to a higher stability of the bacterial communities indicated by a decrease in variation among biological replicates. This was not observed for the susceptible rapeseed lines.

Identification of key phylogenetic groups between mock-treated and infected genotypes associated with resistance to *V. longisporum*

The samples were grouped based on their QTL composition and on their contrasting reactions to *V. longisporum* infection into a partially resistant genotype group consisting of samples from genotypes DH12-R and DH106-R and into a susceptible genotype group consisting of samples from genotypes DH24-S and DH147-S (both groups including 2 genotypes x 3 biological replicated samples each). Within and between these groups different data subsets were compared by t-tests. The differences in relative abundance of phylogenetic groups were compared among the partially resistant and the susceptible genotype groups for the mock-treated and for the *V. longisporum*-infected data sets separately (Tables 2 and 3, on the left). In addition, within each genotype group differences in relative abundance of phylogenetic groups were also compared between the *V. longisporum*-infected versus the mock-treated data sets (Tables 2 and 3, on the right). Discrimination of the statistical associations patterns of these different genotype-treatment-comparisons with phylogenetic group abundance shifts might allow to infer different types of trilateral endophyte-pathogen-host-resistance associations (Tables 2 and 3, columns 7 and 12) as described below.

From the 100 phylogenetic groups exhibiting the highest contribution to differences among samples 44 phylogenetic groups of the root tissue and 55 groups of the hypocotyl tissue showed no association of their relative abundance with resistance/susceptibility genotype groups and were filtered out from analyses. Another 9 and 13 phylogenetic groups of root and hypocotyl tissues showed an association in relative abundance changing between mock-treated and *V. longisporum*-infected genotype groups (infection-induced), but no association with resistance/susceptibility groups. These phylogenetic groups were considered to be enriched in abundance as a consequence of infection and not likely to be involved in a causal relationship with *V. longisporum* resistance and were thus removed from further analyzes. In total, from the 100 phylogenetic groups exhibiting the highest contribution to differences among samples, 47 for root tissue and 32 for hypocotyl tissue showed an association of their relative abundance with resistance/susceptibility genotype groups.

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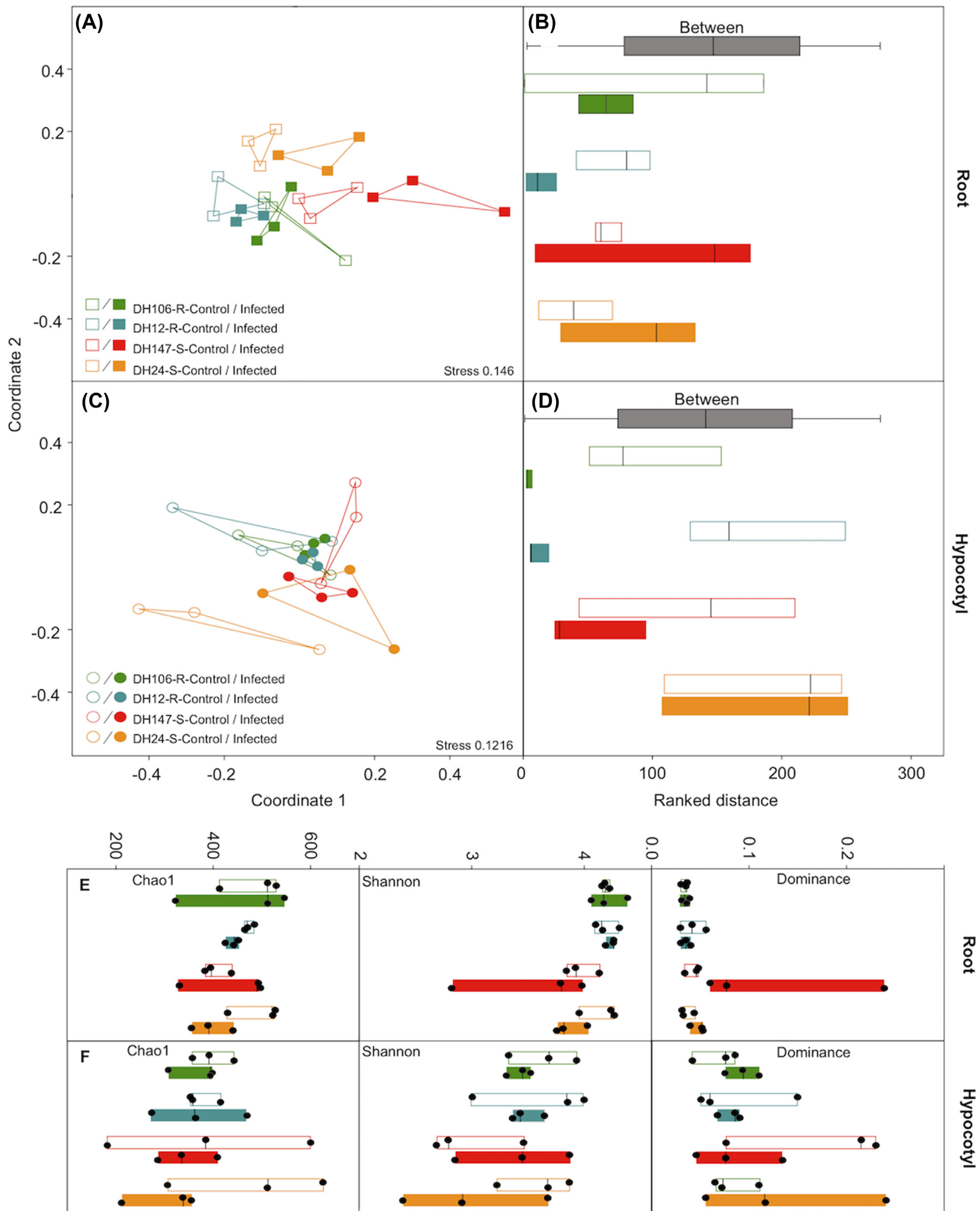

Figure 5. Phylogenetic composition of healthy (mock-treated) and *V. longisporum*-infected roots (above) and hypocotyls (below) of partially resistant rapeseed lines (DH12-R, DH106-R) and susceptible rapeseed lines (DH24-S, DH147-S). **(A, C)**, Non-metric multidimensional scaling (nMDS) analysis of relative abundance pattern of bacterial communities based on the phylogenetic group assignment; **(B, D)**, box plots of ranked distances analyzed at the level of phylogenetic groups; **(E, F)**, box plots of alpha diversity indices Chao 1, Shannon and Dominance index. Analysis A and C were performed in PAST3 and based on a Bray Curtis similarity matrix calculation. Box plots were calculated with the interpolated quartile method. Diversity indices E and F were calculated in PAST3.

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Table 2. Endophytic Bacteria phylogenetic groups showing relative abundance associations among contrasting *V. longisporum* resistance/susceptibility oilseed rape genotype groups in roots.

Taxa ID	Description	Root samples										
		Mock: R vs S		VL-infected: R vs S		Taxa abundance among R and S groups	S: VL-infected vs mock		R: VL-infected vs mock		Taxa abundance among mock and infected groups	Contribution %
		P-value	Fold change	P-value	Fold change	type	P-value	Fold change	P-value	Fold change	type	
T3	*Pseudomonas*	n.s.	0.48	*	0.11	VL-induced	*	3.87	n.s.	0.90	infection-induced in S genotypes	14.61
T1	*Flavobacterium*	n.s.	1.38	**	1.87	VL-induced	n.s.	0.71	n.s.	0.97	constituttive	11.24
T2	*Rhizobium*	**	0.63	**	0.50	constitutive	n.s.	1.05	n.s.	0.83	constitituve	8.97
T23	*Streptomyces*	n.s.	0.56	*	1.53	VL-induced	**	0.21	**	0.56	infection-induced	2.40
T13	*Massilia*	n.s.	1.94	*	0.46	VL-induced	**	3.93	n.s.	0.93	infection-induced in S genotpyes	2.25
T12	*Pelomonas*	n.s.	1.22	**	2.21	VL-induced	n.s.	0.69	n.s.	1.25	constitutive	1.85
T17	uncultured (*Comamonadaceae*)	*	1.97	*	2.13	constitutive	n.s.	0.87	n.s.	0.94	constitutive	1.83
T15	*Pseudoxanthomonas*	n.s.	0.49	*	0.93	VL-induced	n.s.	0.63	n.s.	1.20	constitutive	1.77
T4	*Cellvibrio*	*	0.73	n.s.	0.86	pre-existing	n.s.	0.88	n.s.	1.04	constitutive	1.24
T37	*Actinoplanes*	**	0.56	n.s.	1.64	pre-existing	***	0.22	n.s.	0.63	infection-induced in S genotypes	1.07

VL = *Verticillium longisporum*, R = *V. longisporum* resistant genotype group (DH-12-R, DH-106-R, relative abundance values from 2 × 3 biological replicates), S = *V. longisporum* susceptible genotype group (DH24-S, DH147-S, relative abundance values from 2 × 3 biological replicates), n.s. t-test: P-value is not significant, * t-test: P-value < 0.05, ** t-test: P-value < 0.01, *** t-test: P-value < 0.001, significant associations are shown in grey boxes.

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Table 3. Endophytic Bacteria phylogenetic groups showing relative abundance associations among contrasting *V. longisporum* resistance/susceptibility oilseed rape genotype groups in hypocotyls.

		Hypocotyl samples										
Taxa ID	Description	Mock: R vs S		VL-infected: R vs S		Taxa abundance among R and S groups	S: VL-infected vs mock		R: VL-infected vs mock		Taxa abundance among mock and infected groups	Contribution %
		P-value	Fold change	P-value	Fold change	type	P-value	Fold change	P-value	Fold change	type	
T1	*Flavobacterium*	n.s.	0.87	***	1.48	VL-induced	n.s.	0.74	n.s.	1.25	constitutive	13.47
T3	*Pseudomonas*	n.s.	0.53	*	0.21	VL-induced	n.s.	2.06	n.s.	0.81	constitutive	6.86
T10	*Methylotenera*	*	5.85	n.s.	1.55	pre-existing	*	2.02	n.s.	0.54	infection-induced in S genotypes	2.44
T6	*Caulobacter*	n.s.	0.84	*	1.57	VL-induced	*	0.51	n.s.	0.96	infection-induced in S genotypes	2.08
T9	*Methylophilus*	*	2.48	n.s.	1.46	pre-existing	n.s.	1.32	n.s.	0.78	constitutive	1.97
T8	*Paucibacter*	*	3.76	n.s.	1.36	pre-existing	n.s.	1.16	*	0.42	infection-induced in R genotypes	1.65
T22	*Rheinheimera*	**	3.18	*	2.34	constitutive	*	2.73	*	2.01	infection-induced	1.55
T31	*Leucobacter*	*	0.31	n.s.	0.48	pre-existing	n.s.	0.79	n.s.	1.22	constitutive	1.41
T12	*Pelomonas*	**	5.55	*	1.98	constitutive	n.s.	1.74	n.s.	0.62	constitutive	
T18	*Devosia*	*	0.41	n.s.	1.39	pre-existing	*	0.44	n.s.	1.48	infection-induced in S genotypes	1.05

VL = *Verticillium longisporum*, R = *V. longisporum* resistant genotype group (DH-12-R, DH-106-R, relative abundance values from 2 × 3 biological replicates), S = *V. longisporum* susceptible genotype group (DH24-S, DH147-S, relative abundance values from 2 × 3 biological replicates), n.s. t-test: P-value is not significant, * t-test: P-value < 0.05, ** t-test: P-value < 0.01, *** t-test: P-value < 0.001, significant associations are shown in grey boxes.

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The resistance-associated *Flavobacteria* (T1) and *Pseudomonas* (T3) had the strongest contribution to the differences in the bacterial communities of the root as well as the hypocotyls upon *V. longisporum* infection. Otherwise, between root and hypocotyl samples different phylogenetic groups contributed to the relative abundance and were associated with resistance. The relative abundance of *Flavobacteria* increased in the resistant genotype group upon *V. longisporum* infection whereas the relative abundance of *Pseudomonas* decreased in the resistant genotype group upon *V. longisporum* infection in both, the root (Table 2) and hypocotyl (Table 3) samples. Other resistance-associated groups with high contribution to the community differences among mock plants and *Verticillium*-infected plants were *Rhizobium* (T2), *Streptomyces* (T23), *Massillia* (T13) in root and *Methylotenera* (T10), *Caulobacter* (T6), *Methylophilus* (T9) in hypocotyl tissues. In relative terms, *Rhizobium* (T2) and *Massilia* (T13) decreased in root samples in the resistant genotype group (fold-change below 1 in Table 3 in columns 4 and 6), while *Streptomyces* (T23), *Methylotenera* (T10), *Caulobacter* (T6) and *Methylophilus* (T9) increased in hypocotyl samples in the resistant genotype group (fold-change above 1 in Table 3 in columns 4 and 6).

About half of the resistance-associated phylogenetic groups showed significant differences in relative abundance associated with resistance only after *V. longisporum* infection in the genotype group comparison (e.g. T3, T1, T23, T13, T12, T15 in roots; pairwise T-tests, $P < 0.05$, partial $p>0.001$). Other resistance-associated phylogenetic groups showed significant difference in relative abundance in the mock-treated as well as in the *V. longisporum*-infected genotype group data set comparison (T2 *Rhizobium* and T17 uncultured, family *Comamonadaceae* in roots; T22 *Rheinheimera* and T12 *Pelomonas* in hypocotyls, Table 3). A third minor class of endophytic phylogenetic groups showed significant differences between resistant and susceptible genotype groups only in the mock-treated data set (e.g. T4 *Cellvibrio* and T37 *Actinoplanes* in roots). In addition, for most resistance-associated phylogenetic groups the relative abundance between mock-treated and *V. longisporum*-infected samples in one genotype group (R or S) was not different (Table 3, on the right, phylogenetic group abundance among mock-treated and *V. longisporum*-infected groups: constitutive). Only some phylogenetic groups show an increase in abundance in the mock-treated versus the *V. longisporum*-infected datasets in both or in one of the dataset for the susceptible genotype groups and for the resistant genotype groups. One example for this association pattern is *Streptomyces* (T23) in root tissue. These different types of association patterns in different tissues and treatments are suggesting different modes of direct or indirect interactions of the endophytic bacteria with the host plant or pathogen resulting in efficient infection and/or susceptibility or resistance expression which are discussed below.

DISCUSSION

Microbiological endophytes are known to play an important role in major traits e.g. plant development and growth, pathogen resistance, fitness and diversification or the overall evolution of plants (Hardoim *et al.* 2015).

We found a composition and abundance of endophytic bacteria in roots and hypocotyls of two oilseed rape genotypes dominated by the genera *Flavobacteria* (*Bacteroidetes*) and *Rhizobium* (*Alphaproteobacteria*). This composition broadly corresponds to earlier reports (e.g. Gkarmiri *et al.* 2017 and reviewed by Rathore *et al.* 2019). The composition of endophytic bacteria in healthy root and hypocotyl tissue was found to be partly overlapping. *Flavobacteria*, *Pseudomonas*, *Rhizobium,* and *Cellvibrio* showed the highest contribution to these differences between root and hypocotyl compartments. Although a number of bacterial endophytes with antagonistic properties to *V. longisporum* have been isolated from healthy and infected oilseed rape tissues (Rathore *et al.* 2019), comparative reports on the composition of endophytic bacteria in healthy and infected oilseed rape tissue and their association with resistant and susceptible genotypes for oilseed rape root or leave pathogens are missing. Here we studied if endophyte diversity, composition, and relative abundance pattern of bacterial endophytic communities in oilseed rape is associated with defined quantitative *V. longisporum* resistance expression in selected genotypes.

V. longisporum inoculation of roots of partially resistant and susceptible oilseed rape lines harboring well characterized resistance QTL resulted in clear differences in disease symptom expression and fungal colonization of the hypocotyl tissue measured by qPCR. However, no differences were found for the fungal colonization success of roots in partially resistant and susceptible lines, differences were only found in hypocotyls. This indicates that after successful penetration of the fungus into the epidermal cells of lateral roots of all lines, fungal hyphae are not able to efficiently spread in partially resistant lines into the hypocotyl and colonize the upper portions of the vessel elements and plants. These data confirm the observation that the known quantitative resistance against *V. longisporum* in different oilseed rape accession is resulting in fungal biomass reduction internally within the hypocotyl tissue, but not within the root tissue (Eynck *et al.* 2009). The mechanism underlying this quantitative resistance is still unknown and resistance might be expressed in the root tissue leading to a block in efficient translocation of fungal structures into the hypocotyl tissue in partially resistant genotypes. The present results suggest that endophytic bacteria might be involved in this physical barrier.

In our experiments the *V. longisporum* infection triggered an increase of the total abundance of *Bacteria* in all genotypes and within both tissues, roots and hypocotyls. The experiments also showed a clear genotype-specific influence of *V. longisporum* infection on the *Bacteria* endophytic community diversity and composition among partially resistant and susceptible rapeseed lines. In addition, only in partially resistant lines a shift towards a stabilized relative abundance of certain phylogenetic groups in roots and hypocotyls of partially resistant genotypes among biological replicated samples was observed upon *V. longisporum* infection. This suggests that under *V. longisporum* infection the abundance of certain endophytic *Bacteria* phylogenetic groups is strongly selected in a plant genotype-specific manner controlled by plant genetic host resistance factors and in interaction with the plant pathogen. Resistant and susceptible genotypes might select the propagation of certain endophytic *Bacteria* phylogenetic groups within the tissues. Importantly, for most resistance-associated phylogenetic groups the abundance between mock-treated and *V. longisporum*-infected samples within each of the genotype groups (resistant or susceptible genotype group) is not significantly different (Table 3, column 12, constitutive). This indicates that differences in bacterial abundance associated with resistance/susceptibility are predominantly driven by the genotype groups (resistant or susceptible), and not by the treatment groups (mock- or *V. longisporum*-treated). It is known that the genotype is one important factor in shaping the diversity and abundance of endophytic bacterial communities in roots of oilseed rape (Rathore *et al.* 2019). Here we used sibling lines of a segregating cross with a highly similar background genome and differences in QTL allele

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composition known to be involved in *V. longisporum* resistance expression. The comparisons of these well defined genotype groups suggest that the resistance QTL composition of the genotypes might be involved in shaping the endophyte root and hypocotyl diversity and abundance enhancing resistance to *V. longisporum*, e.g. by promoting *Bacteria* abundance with direct antagonistic effects controlled by host resistance QTL regions. There are a number of studies describing the isolation of *Bacteria* species from the oilseed rape rhizosphere and roots with antagonistic properties to *V. longisporum* or *V. dahliae* (e.g. Berg and Ballin 1994; Pegg and Brady 2002; Granér *et al.* 2003; Berg *et al.* 2005). However, besides promoting direct bacterial antagonism, the putative mechanisms of interactions and associations of certain bacteria species and phylogenetic groups with *V. longisporum* resistance in defined plant genotypes can be explained by a number of models.

For example, the endophytic bacteria phylogenetic groups found in our study to be enriched in abundance and associated with resistance/susceptibility groups might (i) become enriched as a consequence of successful invasion and modification of the tissue by *V. longisporum* in susceptible genotypes (i.e. their involvement in the resistance expression may not be causative); or (ii) become enriched in resistant genotypes because they are directly or indirectly causative for constitutive or induced fungal resistance expression. We found that in some cases the resistance-association is constitutively triggered, while in other cases it is induced after *V. longisporum* infection, or only exists in the healthy genotype group comparison (Table 3, column 7, constitutive, VL-induced, pre-existing).

The first class of resistance-associated phylogenetic groups represents about half of the groups including *Pseudomonas* and *Flavobacteria*, which show differences in relative abundance associated with resistance/susceptibility only after *V. longisporum* infection in the genotype group comparison. This indicates that the abundance shift is induced by or is a consequence of *V. longisporum* infection in resistant/susceptible genotypes. The genus *Pseudomonas* has been reported as one of the most common phylogenetic groups involved in systemic resistance responses against fungal pathogens in a number of crops (Hardoim *et al.* 2015). In *B. napus* more than 5000 rhizosphere bacteria were screened for direct antifungal activity against *V. longisporum* (Pegg and Brandy 2002) and *in-vitro* experiments revealed 17 species, including many *Pseudomonas* ssp. with direct antifungal properties to *V. longisporum*. However, this direct antagonistic interaction is unlikely to play a strong role, as suggested by the *Bacteria* abundance changes in the observed association of the *Pseudomonas* group with *V. longisporum* resistance in our experiments: We found that the relative abundance of *Pseudomonas* bacteria is reduced in the resistant genotype group, (Table 3, fold-change below 1 in columns 4 and 6), but not in the susceptible genotype group. Rather, our results suggest that the association of *Pseudomonas* abundance with *V. longisporum* susceptibility might be due to the prevalence of *Pseudomonas* species pathogenic to oilseed rape within the detected *Pseudomonas* phylogenetic group (e.g. Koike and Kammeijer 2007). In contrast the resistance-associated *Flavobacteria* abundance was found to be higher in the resistant than in the susceptible genotype groups which is consistent with their known biological functioning. Members of the *Flavobacterium* genus are often associated with the capacity to degrade complex organic compounds within plant tissues and their production of extracellular enzymes is believed to be associated with the degradation of bacteria, fungi, insect, and nematode constituents in the environment (Kolton *et al.* 2016).

Another class of phylogenetic groups, representing the other half of resistance-associated phylogenetic groups including *Rhizobium* in roots, was associated in relative abundance with resistance/susceptibility in the mock-treated as well as in the *V. longisporum*-infected data set comparison. This indicated that the phylogenetic group abundance is constitutively different in the resistant genotype group compared to the susceptible genotype group independent of pathogen infection. The phylogenetic groups displaying this class of interactions could be a preferred easy-to-handle target (no pathogen inoculations required) for breeding of resistance-associated endophyte composition in oilseed rape. Surprisingly the abundance of *Rhizobium* bacteria associated with *V. longisporum* resistance/susceptibility in roots was found to be higher in the susceptible genotype group compared to the resistant genotype group although many *Rhizobium* species are considered to exhibit strong direct and indirect plant growth promoting effects inducing the activation of systemic resistances against fungal pathogens (Gopalakrishnan *et al.* 2015). It is known that disruption of host genes involved in *Rhizobium* nodulation in legumes like *M. truncatula* can result in enhanced as well as in decreased susceptibility to soil-borne pathogens including Verticillium wilt (Ben *et al.* 2013) suggesting that plant growth-promoting effects and systemically induced resistances are strongly specific for the trilateral endophyte-pathogen-host interactions and that in *B. napus* the genetically controlled *V. longisporum* resistance is broadly negatively associated with the *Rhizobium* phylogenetic group abundance.

A third minor class of endophytic phylogenetic groups (e.g. *Cellvibrio* in roots) associated with resistance showed statistically significant differences between resistant and susceptible genotype groups only in the mock-treated data set. This indicates that pre-existing differences in relative abundance driven by the genotype groups present in healthy plants exists which disappear after *V. longisporum* infection. For *Cellvibrio* the resistant genotype group is harbouring a higher abundance in healthy plants than the susceptible genotype group. Consistently bacteria from the *Cellvibrio* genus have been described to be involved in carbohydrate degeneration of plant cell walls controlling the development and regulating the activity of plant immunomodulatory function (Li *et al.* 2017).

In conclusion, the comparison of resistance- and treatment-associated differences and abundance patterns of resistance-associated endophytic *Bacteria* groups together with literature-based predominant ecology description of endophyte-pathogen-host group interactions might help to identify resistance-associated *Bacteria* phylogenetic groups. These could be interesting targets for further detailed analyses, and potential microbiome engineering in plant breeding for future *V. longisporum* resistance.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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Conflicts of interest. None declared.

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