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Molecular mechanisms underlying the close association between soil *Burkholderia* and fungi

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Bacterial species belonging to the genus *Burkholderia* have been repeatedly reported to be associated with fungi but the extent and specificity of these associations in soils remain undetermined. To assess whether associations between *Burkholderia* and fungi are widespread in soils, we performed a co-occurrence analysis in an intercontinental soil sample collection. This revealed that *Burkholderia* significantly co-occurred with a wide range of fungi. To analyse the molecular basis of the interaction, we selected two model fungi frequently co-occurring with *Burkholderia*, *Alternaria alternata* and *Fusarium solani*, and analysed the proteome changes caused by cultivation with either fungus in the widespread soil inhabitant *B. glathei*, whose genome we sequenced. Co-cultivation with both fungi led to very similar changes in the *B. glathei* proteome. Our results indicate that *B. glathei* significantly benefits from the interaction, which is exemplified by a lower abundance of several starvation factors that were highly expressed in pure culture. However, co-cultivation also gave rise to stress factors, as indicated by the increased expression of multidrug efflux pumps and proteins involved in oxidative stress response. Our data suggest that the ability of *Burkholderia* to establish a close association with fungi mainly lies in the capacities to utilize fungal-secreted metabolites and to overcome fungal defense mechanisms. This work indicates that beneficial interactions with fungi might contribute to the survival strategy of *Burkholderia* species in environments with sub-optimal conditions, including acidic soils.

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Introduction

Members of the genus *Burkholderia* belong to the class β -Proteobacteria and are widely distributed in the environment. *Burkholderia* are particularly abundant in soil where they can be associated with a wide range of plants (Elliot *et al.*, 2009; Carlier and Eberl, 2012), invertebrates (Kikuchi *et al.*, 2005) and fungi (Warmink *et al.*, 2009; Uroz *et al.*, 2012; Scherlach *et al.*, 2013). Since *Burkholderia* are mostly found in acidic soils (Stopnisek *et al.*, 2014), interactions with fungi might be of particular relevance as fungi also favour acidic environments. From *in vitro* studies, we know that *Burkholderia* can form either antagonistic or mutualistic interactions with fungi. Antagonistic behaviour of *Burkholderia* species is well described and is largely due to

the production of multiple antifungal compounds (Lewenza and Sokol, 2001; Partida-Martinez and Hertweck, 2007; Schmidt *et al.*, 2009) that can be inhibitory to a wide range of phytopathogenic fungi (Quan *et al.*, 2006; Kilani-Feki *et al.*, 2011; Groenhagen *et al.*, 2013). It has been reported that many environmental *Burkholderia* strains have beneficial effects on fungi, suggesting symbiotic and/or mutualistic interactions. Benefits from such interactions were mainly studied in a model system comprised of *B. terrae* and *Lyophyllum* sp., where it was shown that the bacteria (i) colonize the hyphae and use them for transportation and dispersal (Warmink *et al.*, 2011), (ii) survive better in acidic soils (Nazir *et al.*, 2010a) and (iii) use several fungal exudates as nutrients (Warmink *et al.*, 2009; Nazir *et al.*, 2010b, 2013). Along these lines, Drigo *et al.* (2013) reported that *Burkholderia* strains were among the main consumers of carbon released from arbuscular mycorrhizal fungi in a rhizosphere community. In addition, it has been suggested that the presence of fungi is essential for colonization of sterile soils by *Burkholderia* (Nazir *et al.*, 2010a; Warmink *et al.*, 2011).

Despite these reports indicating that *Burkholderia* benefits from partnering with fungi, the following

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questions remain unanswered: (i) how widespread are such associations in soils, (ii) how specific are they, (iii) what is their molecular basis and (iv) what benefits do the bacteria receive from the interaction?

To answer these questions, we designed a comprehensive study in which co-occurrence network analysis, cultivation-based methods and proteomics were used. Co-occurrence network analysis was carried out on soils from a continental-scale study to determine the extent, the specificity and the distribution of such interactions in the soil. Model *Burkholderia* strains previously identified as major soil inhabitants (Stopnisek *et al.*, 2014) were tested further for their ability to translocate and disperse with fungi. Finally, the widespread soil inhabitant *B. glathei* was grown alone or in the presence of either *Fusarium solani* or *Alternaria alternata* and the bacterial proteome was analysed under each situation to identify the molecular and physiological basis of the interaction.

Materials and methods

Co-occurrence analysis

We analysed the microbial communities of 266 soil samples from the Nutrient Network (NutNet) globally distributed experiment (Borer *et al.*, 2014). Details of this data set and the methods used to characterize the bacterial and fungal communities are available in Prober *et al.* (2015). In total, 37 393 ITS fungal operational taxonomic units (OTUs) and 223 693 16S rRNA bacterial OTUs (with OTUs defined at >97% sequence similarity level for ITS1 and 16S rRNA reads for fungi and bacteria, respectively) were included to the analysis. Co-occurrence patterns between *Burkholderia* and fungal OTUs were tested using Spearman's rank correlations between OTUs that occurred in at least 5% of the samples and had a rho of >0.3 and *P*-value <0.01 adjusted using the false discovery rate method (Barberan *et al.*, 2012).

Monitoring the interactions between fungi and *Burkholderia* sp.

Fungi used in this experiment belonged to the species *Alternaria alternata*, *Fusarium solani*,

Rhizoctonia solani and *Lyophyllum* sp. Karsten, while *Burkholderia* species applied were *B. glathei*, *B. hospita*, *B. fungorum* and *B. caledonica* (Table 1). To visualize the interaction with fungi under the fluorescent microscope, all *Burkholderia* strains were tagged with either the green fluorescent protein (GFP) or the dsRED protein using the electroporation protocol described by Choi *et al.* (2006). In short, overnight cultures of *Burkholderia* strains were washed twice in 0.3 M sucrose and subsequently electroporated with the plasmid pBBR1MCS-2-gfp mut3 Km^r or pin62: DsRed Cm^r. Electroporated cells were transferred on *Pseudomonas* isolation agar and Luria-Bertani (LB) plates with the corresponding antibiotics (50 mg l⁻¹). To follow the interactions, fungi were inoculated on water agar plates (15 g l⁻¹) or LB agar at least 3 days before spotting the bacteria on the mycelium. For that overnight liquid *Burkholderia* cultures were prepared, washed three times in 0.9% NaCl, OD₆₀₀ was adjusted to 0.1 and 4 drops of 2 µl were spotted on the fungal mycelium pre-grown for 3 days. Plates were incubated for up to 10 days in the dark and the growth of *Burkholderia* as well as interactions with fungi were followed daily under the fluorescence microscope (Leica M165 FC, Mannheim, Germany) and binocular (Leica DM6000 B, Mannheim, Germany). To monitor dispersal and attachment of *Burkholderia* strains on hyphae, a sterile iron ring was placed before pouring in the middle of the plate, which served as a physical barrier for bacterial cells. Both sides were filled up to 5 mm below the edge of the iron circle. Selected fungi and *Burkholderia* strains were inoculated in the inner side of the ring, so that only bacterial cells which could attach and/or disperse would be detected on the outer side of the ring. All experiments were performed in triplicates.

Proteome analysis

For proteomic analyses, *B. glathei* LMG14190 and two fungi, *A. alternata* and *F. solani*, were used. Co-cultures with *A. alternata* (AB) and *F. solani* (FB) as well as the *B. glathei* alone (B) were grown in triplicates as described above with some modifications: as growth medium 1/3 Difco Malt Extract

Table 1 Bacterial and fungal strains used in this study

Strains	Application	References
<i>Burkholderia fungorum</i> LMG16225	Interaction studies	Coenye <i>et al.</i> (2001)
<i>Burkholderia glathei</i> LMG14190	Interaction studies, genome sequencing, proteomics	Vandamme <i>et al.</i> (1997)
<i>Burkholderia hospita</i> LMG20598	Interaction studies	Goris <i>et al.</i> (2002)
<i>Burkholderia phytofirmans</i> LMG22487	Interaction studies	Sessitsch <i>et al.</i> (2005)
<i>Alternaria alternata</i>	Interaction studies, proteomics	Phytopathology group
<i>Fusarium solani</i>	Interaction studies, proteomics	Institute of Plant Sciences
<i>Rhizoctonia solani</i>	Interaction studies	Federal Institute of Technology, Zurich, Switzerland
<i>Lyophyllum</i> sp. Karsten DSM 2979	Interaction studies	Leibniz Institute DSMZ

Agar (MEA) (BD, Franklin Lakes, NJ, USA) with 7 g agar l⁻¹ medium was used and plates were supplemented with a cellophane membrane (Bio-Rad, Hercules, CA, USA; 165-0963-MSDS). After 10 days at room temperature, the biomass was collected and samples were treated as described by Carlier and Eberl (2012) with some modifications: to separate bacterial and fungal cells, mixed samples were homogenized in phosphate-buffered saline solution with a pestle, debris were pelleted at 1000 g for 3 min and the top layer was transferred to 50% Percoll (Sigma-Aldrich, St Louis, MO, USA). After ultracentrifugation at 15 000 g for 3 h, fractions were collected and analysed by phase-contrast light microscopy (Leica DM6000 B) and bacterial fractions were pooled and washed twice in phosphate-buffered saline solution. To extract proteins, a bead-beating protocol was used. Fractions were transferred into 2 ml tubes (Sarstedt, Nürnbrecht, Germany) with 0.25 ml of 0.1 mm glass beads (BioSpec Products, Bartlesville, OK, USA) and placed into FastPrep-24 lyser tubes (MP Biomedicals, Santa Ana, CA, USA). Eight rounds of 30 s lysis of 6.5 ms⁻¹ followed by 5 min incubation on ice were used to extract proteins in concentrations from 2 to 8 mg ml⁻¹. In all, 25 µg of cytosolic protein extract (Roti-Nanoquant, Roth, Karlsruhe, Germany) was resolved on 1D SDS-PAGE, lanes were cut in equidistant pieces and in-gel digested with trypsin as recently described (Grube *et al.*, 2014). Peptide mixtures were separated by RP chromatography using an EASYnLC 1000 (Thermo Fisher Scientific, Waltham, MA, USA) with self-packed columns. Peptides were loaded and desalted on the separating column following resolution with a binary non-linear 76 min-gradient from 5% to 75% ACN in 0.1% acetic acid at a constant flow rate of 300 nl min⁻¹. The liquid chromatography system was coupled online to an Orbitrap Elite equipped with a nanoelectrospray ion source (Thermo Fisher Scientific) performing mass spectrometry (MS) and MS/MS experiments of the eluted peptides. Survey scans were recorded in m/z range from 300 to 1700 with a resolution of 60 000 and with lock mass option enabled. MS/MS experiments were performed for the 20 most intensive precursor ions as determined in the survey scan excluding unassigned charge states or singly charged ions from the MS/MS experiments in the Linear ion trap. Proteins were identified by searching all MS/MS spectra against a database containing protein sequences of *Burkholderia glathei* LMG14190, *Fusarium solani* and *Alternaria alternata* (version 13-Dec-17) with added laboratory contaminants using Sorcerer-SEQUEST (Sequest v. 27 rev. 11, Thermo Scientific) including Scaffold 4.0.5 (Proteome Software, Portland, OR, USA). SEQUEST was searched with a parent ion tolerance of 10 ppm and a fragment ion mass tolerance of 1.00 Da. Up to two missed tryptic cleavages were allowed. Methionine oxidation (+15.99492 Da) was set as variable modification. For protein identification, a stringent SEQUEST filter for peptides was used (Xcorr versus

charge state: 2,2, 3,3 and 3,8 for doubly, triply and quadruply charged peptides and deltaCn value greater than 0.10) and at least two peptides per protein were required. Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. The protein false discovery rate, based on a decoy database, was 0.4%. Relative quantification was based on spectral counting of exclusive spectra using normalized spectral abundance factors (Zhang *et al.*, 2010). Statistical analysis using MeV v4.8.1 (Saeed *et al.*, 2003) was done for proteins that were present in at least two out of three biological replicates. Hierarchical clustering and Student's *t*-test of z-transformed normalized exclusive spectra were performed with the following settings: unequal group variances were assumed (Welch approximation), *P*-values based on permutation (1000) with *P*=0.05, significance determined by standard bonferroni correction. Only proteins showing at least twofold changes in addition to statistical significance were considered for further analysis. So-called 'off/on' proteins needed to be detected in all replicates of one treatment and absent from all replicates of another treatment. Functional prediction and assignment of proteins to cluster of orthologous groups and TIGRFAMs (Haft *et al.*, 2013), respectively, was accomplished by the in-house developed analysis pipeline 'Prophane 2.0' (<http://www.prophane.de>; Schneider *et al.*, 2011). Voronoi treemaps were generated using Paver (Decodon, Greifswald, Germany, <http://www.decodon.com/>).

Burkholderia glathei LMG14190 genome sequencing

High molecular mass DNA was extracted following a standard protocol (Wilson 2001). Paired-end insert libraries were constructed at the FGCZ (Functional Genomics Center Zurich) using the Nextera XT kit (Illumina, San Diego, CA, USA). Sequencing of the 2 × 250 bp inserts was performed on the Illumina MiSeq platform at the FGCZ. The sequencing reads were assembled using the SPAdes v2.5 software (Nurk *et al.*, 2013). The resulting 138 contigs were examined and edited for misassemblies in GAP5 (Bonfield and Whitwham, 2010). Ribosomal RNA and tRNA genes were predicted using RNAmmer and tRNAscan (Schattner *et al.*, 2005; Lagesen *et al.*, 2007). The predicted RNA genes were masked using the maskfeat utility of the EMBOS software package (Rice *et al.*, 2000) before protein-coding gene finding using the Prodigal software (Hyatt *et al.*, 2010). The annotated contigs, containing CDS and RNA gene predictions, were submitted to the RAST online service for functional annotation (Aziz *et al.*, 2008). The annotations were curated using the Artemis software suite (Carver *et al.*, 2008). The annotated sequences were deposited at the European Nucleotide Archive (accession numbers CCNS01000001–CCNS01000139).

Results and Discussion

Burkholderia co-occur with fungi in soils

The frequency of fungi co-occurring with *Burkholderia* was assessed with co-occurrence analysis from a trans-continental soil sample collection. The analysis supported our hypothesis that members of the genus *Burkholderia* significantly co-occur with multiple fungal taxa, suggesting broad and frequent associations between fungi and *Burkholderia* in soils (Figure 1, Supplementary Table S1). Apart from *Burkholderia*, other bacterial genera also showed high co-occurrence with fungi. Whether these patterns reflect an intimate interaction with the fungi or result from their shared preference for low pH or other environmental parameters remains to be investigated. For *Acidobacteria* and *Verrucomicrobia*, two phyla containing OTUs that showed high co-occurrence with fungi, low pH dependency has been demonstrated but to our knowledge there is no evidence for beneficial interactions between members of those phyla and fungi. In contrast, while the genus *Burkholderia* also shows strong preference for acidic soils (Stopnisek *et al.*, 2014), mounting evidence suggests that many *Burkholderia* species establish direct associations with fungi and may often live directly on or inside fungal cells (Warmink *et al.*, 2011; Nazir *et al.*, 2012; Uroz *et al.*, 2012). Thus, we speculate that the strong co-occurrence of *Burkholderia* and fungi indicates that the two organisms interact with each other in the soil, rather than just reflecting shared niche preferences.

Burkholderia sp. attach, translocate and disperse on fungal hyphae

To test the specificity of the interactions and the ability of *Burkholderia* to attach, translocate or disperse on hyphae of different fungal species, we designed an *in vitro* experiment. Four *Burkholderia* strains that were previously identified as widespread soil inhabitants (Stopnisek *et al.*, 2014), and four fungi that were among those taxa which most frequently co-occurred with *Burkholderia* were grown in dual cultures in Petri dishes containing

Figure 1 Co-occurrence of fungal (grey boxes) and *Burkholderia* (black boxes) OTUs from 266 soil samples. Box size represents the number of significant ($\rho > 0.3$, P -value < 0.01 after false discovery rate (FDR)) associations for each OTU.

an iron ring (Figure 2). The fungus and the bacteria were inoculated together inside the ring and only those bacteria able to attach to the hyphae were able to cross the ring to reach the outer compartment. This experiment was carried out both with water agar, on which bacteria alone could not grow, and with LB as growth media in the outer compartment. Interestingly, even when the rich LB medium was used, *Burkholderia* strains were mainly found in close vicinity to fungal hyphae, suggesting that the benefits provided by the fungi were not restricted to transportation, but included other goods that caused bacteria to stay on the hyphae rather than spread onto the rich medium. Our experiments showed that (i) for all four tested *Burkholderia* strains, growth on the hyphae was favoured over growth on the medium (Figure 2) and (ii) all tested *Burkholderia* strains were able to cross the iron ring and be translocated with the hyphae of the fungi tested, with the exception of *Lyophyllum* sp. Karsten. Under the conditions used, the mycelium of *Lyophyllum* showed hydrophobicity, thus inhibiting the formation of a water film around the hyphae, which might be necessary for bacterial attachment and dispersal (Pion *et al.*, 2013). However, Warmink *et al.* (2011) showed that *B. terrae* BS001 could migrate together with *Lyophyllum* sp. Karsten in soil microcosms,

Figure 2 Monitoring *Burkholderia*-fungi interactions. Plates with sterile iron rings and either water agar, LB or a combination of both were used to monitor the ability to translocate, to disperse and to form biofilms on the hyphae. For microscope observations, *Burkholderia* cells were tagged with gfp or DsRed (on the pictures *B. glathei* LMG14190 tagged with DsRed), co-cultivated with fungi (here *F. solani*) and monitored on a daily basis. Microscopy pictures represent different stages of dispersal on *F. solani* hyphae over time. (a) Network of hyphae is densely colonised by bacteria (red) in the inner part of the ring. (b) Crossing of the iron ring by fungal hyphae enables *Burkholderia* cells to cross too when attached to the hyphae. (c) Colonization of the hyphae by transported *Burkholderia* occurs also on the outer part of the iron ring. The same behaviour was visible when water agar was replaced by LB.

suggesting that the physiology of this fungus may change when growing in soil. Our results are consistent with the results reported by Nazir *et al.* (2012), who found that 19 *Burkholderia* strains were able to co-migrate with *Lyophyllum* sp. Karsten, but their abilities differed depending on the soils used. No visible deleterious effects of the bacteria on mycelial growth or morphology were observed.

Global analyses to elucidate the molecular basis of *Burkholderia*-fungi interactions

We used a proteomic approach to characterize the molecular nature of the interaction between *Burkholderia* strains and fungi. Among the fungal OTUs that co-occurred with *Burkholderia*, those belonging to the genera *Alternaria* and *Fusarium* were among the most frequently detected ones in our analysis (Supplementary Table S1), and thus we selected *A. alternata* and *F. solani* as model species. As a representative of the genus *Burkholderia*, *B. glathei* LMG14190 (T) (Vandamme *et al.*, 1997) was chosen for these interaction studies, since members of this species were not only the most frequently detected in a previous soil survey (Stopnisek *et al.*, 2014), but have also been demonstrated to interact with fungi (Koele *et al.*, 2009).

Genetic features of *B. glathei* LMG14190 possibly involved in interactions with fungi

To obtain insights into the reasons for the ubiquitous distribution of *B. glathei* in soils, as well as for better interpretation of proteomics data, we sequenced the genome of *B. glathei* LMG14190, which was not available at the time of our analyses. The draft genome is 8'049'485 bp in size, which is consistent with already sequenced strains of the genus *Burkholderia* (Xu *et al.*, 2013; Carlier *et al.*, 2014; Liu *et al.*, 2014). Annotation of the draft genome predicted 7216 protein coding genes, 50 tRNAs and 3 rRNAs (PRJEB6934).

The genome of *B. glathei* LMG14190 encodes a large number of genes involved in transport and utilization of various carbon sources. In addition to the utilization of simple sugars, *B. glathei* LMG14190 has the genetic potential to degrade polymeric carbohydrates such as chitin, cellulose, glycogen, as well as alcohols, including ethylene glycol, ethanol and butanediol. *B. glathei* LMG14190 contains genes coding for enzymes involved in the degradation of glycerol, which is predicted to be one of the C sources fungi secrete (Nazir *et al.*, 2013). Additionally, fungi produce a number of aromatic compounds that could represent another source of nutrients for *Burkholderia* (Gutiérrez *et al.*, 1994). Degradation of aromatic compounds is a commonly observed feature of various *Burkholderia* strains (Pérez-Pantoja *et al.*, 2012; Andreolli *et al.*, 2013; Schamfuss *et al.*, 2013; Xu *et al.*, 2013) and the corresponding genes were also identified in the

genome of *B. glathei* LMG14190. Beside pathways for the utilization of toluene, phenolic, benzoate and catechol compounds, the genome also encodes the complete salicylate degradation pathway. Given the antibacterial and antifungal properties of many of these aromatic compounds, the capacity to degrade such substances could also serve as a protection mechanism for the bacteria and/or their fungal partners.

The type three secretion system has been described as essential to the interaction between *B. terrae* BS001 and *Lyophyllum* sp. Karsten (Warmink and van Elsas, 2008). However, homologous genes could not be detected in the genome of *B. glathei* LMG14190. We also tested for the presence of the *hrcR* gene, a highly conserved component of the type three secretion system (Warmink and van Elsas, 2008), in the four *Burkholderia* strains used in our dual culture assays. Surprisingly, the *hrcR* gene could only be amplified from one of the strains tested, *B. hospita*, but was not detected in *B. glathei*, *B. fungorum* or *B. phytofirmans* (data not shown). This suggests that type three secretion system is not essential for the interaction with the fungi tested here. Nonetheless, the genome of *B. glathei* LMG14190 harbours genes that have been described to have a role in biological interactions, such as those involved in the synthesis of the exopolysaccharide cepacian (BGLT00414-BGLT00424), which is produced by most *Burkholderia* species and is important for interactions with other organisms as well as for resistance to different stresses (Ferreira *et al.*, 2011).

Global changes in the proteome of *B. glathei* when co-cultivated with fungi

A total of 2406 unique *B. glathei* LMG14190 proteins and only 3 fungal proteins were detected in the present study, indicating an efficient enrichment of bacterial cells from the co-cultures (Supplementary Table S2). After applying stringent quality filters and considering only proteins identified in at least 2 out of 3 biological replicates, 1583 proteins remained, the expression of which could be semi-quantitatively assessed by spectral counting (Supplementary Table S3). Among those, 923 proteins (almost 60%) were identified in both, single and co-cultures, while 282, 47 and 29 proteins were found to be exclusively expressed during single culturing or co-cultivation with *A. alternata* and *F. solani*, respectively (Figure 3). Overall, 742 were differentially expressed (expression change of more than twofold) during co-cultivation compared to the single cultures with the majority of these proteins expressed in lower amounts in the presence of either of the fungi. Most of the proteins identified in this study were assigned to functions associated with the production and conversion of energy, as well as with transport and metabolism of carbohydrates and amino acids (Supplementary Table S2). This corresponds to the high proportion

Figure 3 Venn diagram depicting quantified proteins of *B. glathei* LMG14190 grown in single culture (B) and in co-cultivation with *A. alternata* (BA) and *F. solani* (BF), respectively.

of predicted genes with the same function in the genome of *B. glathei* LMG14190 (approximately 20% of the whole genome). In contrast, functional classes such as transcription, defence and signalling were under-represented in the *B. glathei* proteome when considering their contribution to the total number of predicted genes (Supplementary Table S2).

To shed light on the molecular mechanisms involved in bacterial–fungal interactions, we focussed mainly on the *B. glathei* proteins whose abundance changed significantly during fungal co-cultivation. As shown in Figures 4 and 5 co-cultivation affected protein expression across all functional categories. Notably, a particular high percentage of differentially expressed proteins were involved in cell motility and translation (Figures 4 and 5). The putative relevance of the observed protein expression changes during co-cultivation to the biological interaction between *B. glathei* and fungi is highlighted below.

Attenuation of starvation and stress responses in *B. glathei* when co-cultivated with fungi

B. glathei grown in the absence of fungi was limited to the nutrients available in the growth medium (1/3 MEA) and this resulted in the expression of many transcriptional regulators related to starvation and stress response. The expression rates of most of these regulators and stress factors were significantly reduced in the co-cultivations, suggesting multiple beneficial effects of fungi on *B. glathei* (Supplementary Table S3). From a total of 255 predicted proteins in signal transduction mechanisms, 36 and 32 were present in co-cultivations with *A. alternata* (AB) and *F. solani* (FB), respectively, with 29 of them shared between the co-cultivations (Supplementary Table S3), suggesting similar metabolic response and regulation of *B. glathei* when growing with both fungi. Most of these proteins were factors related to stress or starvation linked to important nutrients such as nitrogen, phosphate and carbon, which were all relieved upon co-cultivation. For instance, the attenuation of nitrogen (N) starvation

in both co-cultivations was supported by the decrease of the nitrogen regulation protein NR_I (BGLT03875) and of the glutamine synthetase (BGLT01582). The nitrogen regulation protein NR_I is the major activator of nitrogen-controlled genes such as the glutamine synthetase (Reitzer and Magasanik, 1985; Reitzer *et al.*, 1989). It has been shown that nitrogen starvation in *E. coli* leads also to an increase of proteins involved in amino acid and polyamine degradation (Zimmer *et al.*, 2000). Decreased expression of various amino-acid transport systems as well as of amino acid and polyamine degradation pathways in *B. glathei* in co-cultivations additionally supports the idea that the fungi alleviate the N starvation response (Figures 4 and 5). Even though most of the pathways and transport systems showed lower abundances compared with the single culture, subunits of histidine ABC transporters (BGLT00146, BGLT01156) were significantly increased in co-cultivations. Histidine could act as N source provided by fungi to *B. glathei*, since many enzymes of the histidine degradation pathway, such as histidine ammonia-lyase (BGLT04159), urocanate hydratase (BGLT03664) and N-formylglutamate deformylase (BGLT04163), were detected (Supplementary Table S3).

Beside N starvation, phosphate (P) starvation seems to have been alleviated in co-cultivation, as indicated by a significant decrease or absence of proteins such as phosphate starvation-inducible protein (BGLT03521) and glycerol-3-phosphate uptake system (BGLT04691, BGLT05290, BGLT00157) in the co-cultivation with fungi, compared with the control. Expression of proteins associated with the UgpBAEC glycerol-3-phosphate uptake system, a member of the ABC superfamily, was among the most strongly decreased in the co-cultivation experiments. In *E. coli*, glycerol-3-phosphate can be used as C and/or P source and is also an essential intermediate in phospholipid biosynthesis (Schweizer *et al.*, 1982; Boos, 1998). Glycerol-3-phosphate is an insufficient carbon source, but a sufficient P source for *E. coli* (Boos, 1998). Thus, the Ugp system that is used for scavenging phosphate-containing compounds in *E. coli* may also serve this purpose in *Burkholderia*.

The limited amount of carbon contained in the growth medium could explain the detection of proteins indicative of C starvation in the pure culture control. In both co-cultivations, however, most of these proteins were absent or strongly decreased, such as the Stringent starvation protein A, SspA (BGLT04645) for instance. In *E. coli*, SspA expression is induced by starvation for glucose, nitrogen, phosphate, amino acids as well as by a decreased growth rate (Williams *et al.*, 1994). In addition to SspA, the carbon starvation protein A, CstA (BGLT04027), a protein that is more specifically expressed during C starvation was also detected and significantly decreased in expression when *F. solani* was present, while no change was detected in co-cultivation with *A. alternata*. The decreased amount of SspA and other C starvation proteins

Figure 4 Impact of co-cultivation on proteome composition of *B. glathei* LMG14190. The percentage of proteins in relation to the theoretical proteome with at least twofold change in co-cultivation with either fungus compared with the control (*B. glathei* alone) is depicted. BA, co-cultivation of *B. glathei* with *A. alternata*; BF, co-cultivation of *B. glathei* with *F. solani*. Black bars indicate proteins with changed amount in both co-cultivations, white bars indicate proteins with changed amounts in the BF co-cultivation, grey bars indicate proteins with changed amounts in the BA co-cultivation. Functional annotation is based on cluster of orthologous groups (COG).

during co-cultivation suggests a preference of *B. glathei* for fungal exudates over the C source present in the growth medium (MEA), which is mainly maltose. Indeed, the maltose ABC transporter (BGLT01992), which is responsible for the uptake of maltose, was decreased in co-cultivations. Additionally, a xylose ABC transporter (BGLT06434) was also strongly decreased in co-cultivation. D-xylose is the most abundant sugar in nature after glucose (Ladisch *et al.*, 1983), and it can be utilized by *E. coli* as a sole carbon source and metabolized through the pentose phosphate pathway (Song and Park, 1998). That xylose was used when *B. glathei* grew alone on MEA is suggested by the expression of xylose isomerase (BGLT06433), which catalyses the first reaction in the catabolism of D-xylose (Schellenberg *et al.*, 1984) and which was not present in the co-cultivations with either fungus (Supplementary Table S3).

While our results suggest that *B. glathei* may preferentially utilize fungal exudates in the co-cultivation experiments, we could not unambiguously identify the primary C source of *B. glathei* in the exudates. A likely candidate appears to be ribose, as the amounts of proteins involved in ribose uptake

(BGLT04604, BGLT04606 and BGLT05567) were strongly increased in co-cultivation.

Burkholderia proteins involved in detoxification processes in the presence of both fungi

In contrast to the positive effects of fungi on alleviation of nutrient starvation, proteins indicating stress were also detected when *B. glathei* was grown with the fungi. An increase in various catalases and peroxidases (BGLT01132, BGLT04737) in co-cultivations suggests that bacteria experienced oxidative stress when grown with fungi (Supplementary Table S3). Increased expression of 5-methyltetrahydropteroyltrimethylglutamate homocysteine methyltransferase, MetE (BGLT01007) in co-cultivation with *A. alternaria* also suggests oxidative stress, since this protein is very highly expressed in *E. coli* cells exposed to oxidative stress (Hondorp and Matthews, 2004). Antibacterial compounds released by the fungi could cause oxidative stress (Ammar *et al.*, 1979; Hellwig *et al.*, 2002; Deshmukh *et al.*, 2014; Soltani and Hosseini Moghaddam, 2014). An increase of the RND (resistance nodulation division) family of efflux pumps

Figure 5 Voronoi treemaps visualizing changes in the proteome composition of *B. glathei* LMG14190 during fungal co-cultivation. Expression ratios of *B. glathei* LMG14190 proteins in single cultures (B) compared to co-cultivation with *A. alternata* (AB) or *F. solani* (FB). Functional classification of proteins was carried out by Prophane 2.0 and is based on cluster of orthologous groups (COG). Red colour indicates higher expression of the respective protein in single cultivation and blue colour indicates higher expression in co-cultivations. Each cell represents a single protein, functional classes are separated by thicker black lines.

(BGLT02584, BGLT02585, BGLT02586, BGLT04622, BGLT05365) in co-cultivations further supports the idea of fungal release of antibacterial molecules. The detected proteins show high homology to members of the NodT family of outer membrane transport proteins from the genus *Rhizobium* (Rivilla *et al.*, 1995) and the CmeABC multidrug efflux system of *Campylobacter jejuni* (Lin *et al.*, 2002), which are involved in the export of a wide range of drugs. In addition to drug resistance, RND pumps have an important role in biological interactions such as nodulation, colonization and host persistence (Piddock, 2006). In *B. cenocepacia*, RND efflux pumps also influence phenotypic traits involved in pathogenesis, such as motility and chemotaxis (Bazzini *et al.*, 2011). In addition to these detoxification systems, other proteins with a putative function in detoxification were found to be increased in co-cultivations: the proteins YagS (BGLT01120), YagT (BGLT01121) and YagR (BGLT00865), which are part of the *yagT*SRQ operon, were significantly increased in both co-cultivations (Supplementary Table S3). This operon was shown to encode a periplasmatic aldehyde oxidoreductase that oxidizes a broad spectrum of aldehydes to their respective acids, thereby contributing to the detoxification of the cells (Neumann *et al.*, 2009).

RND efflux pumps were not the only proteins that were increased and that are associated with cell envelope biogenesis and outer membrane structures: many outer membrane porines and exporters

(BGLT02029, BGLT00225, BGLT02300, BGLT00513 and BGLT04032) were also highly increased in co-cultivations. Although the functions of most of these proteins remain unknown, a recent transcriptomic study of *B. cenocepacia* J2315 stress responses revealed that most of the homologous proteins were increased under nutrient starvation and low oxygen conditions, or when exposed to oxidative stress (Sass *et al.*, 2013). Thus, the expression of membrane-associated proteins observed in co-cultivation might be due to stresses induced by the fungi, although changes in membrane composition could also originate from osmotic stress. The primary response to osmotic stress in most bacteria is the uptake of potassium (Paul 2013). Interestingly, the two homologous potassium uptake systems (BGLT02399-BGLT02401 and BGLT06537-BGLT06539) encoded in *B. glathei* genome were expressed in both co-cultivations (Supplementary Table S3).

B. glathei proteins involved in motility are not expressed in fungal co-culture

Using fungal hyphae for dispersal has been shown in previous studies to be advantageous in the soil, where heterogeneity of soil particles and the lack of water films represent major obstacles to motility. In our *in vitro* experiments, dispersal of *Burkholderia* strains on fungal hyphae was observed but many proteins involved in motility, which were expressed in the control, were undetected in the presence of the

Figure 6 Schematic overview of the most important changes in *B. glathei* LMG14190 proteome during co-cultivation with *A. alternata* and *F. solani*. The overview includes proteins involved in major pathways that have been detected in our analysis and proteins whose amounts were either decreased (red) or increased (green) in both co-cultivations. Dashed lines represent the effects of hypothetical stress factors (lightning symbols) or substrates that could be produced by fungi. All flagellar proteins were absent in the co-cultivation setups, thus the whole flagella apparatus is marked in red.

two fungi (Figures 4 and 5, Supplementary Table S3). This might indicate that bacterial cells encountering a fungal hypha cease to invest resources into their own motility and rather rely on the fungus for transport.

Conclusion

Evidence has emerged over the past years that *Burkholderia* are often associated with fungi in soils (Warmink et al., 2009; Lepleux et al., 2012; Nazir et al., 2012). The co-occurrence analysis presented here fully supports this notion and extends our understanding of the ability of *Burkholderia* to form associations with a broad range of fungal taxa. The widespread soil inhabitants *Burkholderia glathei*, *B. terrae*, *B. fungorum* and *B. phytofirmans* could all interact and disperse with *A. alternata*, *F. solani* and *R. solani*, suggesting that these broadly occurring interactions might also occur in the environment. Previous reports indicated that the interactions with fungi might be beneficial for the bacteria, yet knowledge of the nature of these benefits is still scarce (Nazir et al., 2010a, 2013; Warmink et al., 2011). Our proteome analysis, which gave a snapshot view of a dynamic interaction, revealed that in a

nutrient-limited medium, *B. glathei* was able to use multiple substrates provided by the fungi, which attenuated the starvation response observed when grown alone (Figure 6). However, *B. glathei* encountered new stresses when co-cultivated with fungi, as revealed by the higher expression of proteins associated with various defence and tolerance mechanisms. These functions seem important for the successful colonization and persistence of *Burkholderia* sp. on fungal hyphae. The benefits that *Burkholderia* appear to gain from the interaction with fungi are likely to outweigh the costs involved in the co-existence and thus these associations might represent a successful strategy to permit the survival of *Burkholderia* in the soil environment.

Conflict of Interest

The authors declare no conflict of interest.

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References

- Ammar MS, Gerber NN, McDaniel LE. (1979). New antibiotic pigments related to fusarubin from *Fusarium solani* (Mart.) Sacc. I. Fermentation, isolation, and antimicrobial activities. *J Antibiot* **32**: 679–684.
- Andreolli M, Lampis S, Poli M, Gullner G, Biro B, Vallini G. (2013). Endophytic *Burkholderia fungorum* DBT1 can improve phytoremediation efficiency of polycyclic aromatic hydrocarbons. *Chemosphere* **92**: 688–694.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA et al. (2008). The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* **9**: 75.
- Barberan A, Bates ST, Casamayor EO, Fierer N. (2012). Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J* **6**: 343–351.
- Bazzini S, Udine C, Sass A, Pasca MR, Longo F, Emiliani G et al. (2011). Deciphering the role of RND efflux transporters in *Burkholderia cenocepacia*. *PLoS ONE* **6**: e18902.
- Bonfield JK, Whitwham A. (2010). Gap5—editing the billion fragment sequence assembly. *Bioinformatics* **26**: 1699–1703.
- Boos W. (1998). Binding protein-dependent ABC transport system for glycerol 3-phosphate of *Escherichia coli*. *Methods Enzymol* **292**: 40–51.
- Borer ET, Harpole WS, Adler PB, Lind EM, Orrock JL, Seabloom EW et al. (2014). Finding generality in ecology: a model for globally distributed experiments. *Methods Ecol Evol* **5**: 65–73.
- Carlier A, Agnoli K, Pessi G, Suppiger A, Jenul C, Schmid N et al. (2014). Genome sequence of *Burkholderia cenocepacia* H111, a cystic fibrosis airway isolate. *Genome Announc* **2**: pii: e00298-14 (1–2).
- Carlier AL, Eberl L. (2012). The eroded genome of a *Psychotria* leaf symbiont: hypotheses about lifestyle and interactions with its plant host. *Environ Microbiol* **14**: 2757–2769.
- Carver T, Berriman M, Tivey A, Patel C, Bohme U, Barrell BG et al. (2008). Artemis and ACT: viewing, annotating and comparing sequences stored in a relational database. *Bioinformatics* **24**: 2672–2676.
- Choi K-H, Kumar A, Schweizer HP. (2006). A 10-min method for preparation of highly electrocompetent *Pseudomonas aeruginosa* cells: Application for DNA fragment transfer between chromosomes and plasmid transformation. *J Microbiol Methods* **64**: 391–397.
- Coenye T, Laevens S, Willems A, Ohlén M, Hannant W, Govan JR et al. (2001). *Burkholderia fungorum* sp. nov. and *Burkholderia caledonica* sp. nov., two new species isolated from the environment, animals and human clinical samples. *Int J Syst Evol Microbiol* **51**: 1099–1107.
- Deshmukh R, Mathew A, Purohit HJ. (2014). Characterization of antibacterial activity of bikaverin from *Fusarium* sp. HKF15. *J Biosci Bioeng* **117**: 443–448.
- Drigo B, Kowalchuk GA, Knapp BA, Pijl AS, Boschker HTS, van Veen JA. (2013). Impacts of 3 years of elevated atmospheric CO₂ on rhizosphere carbon flow and microbial community dynamics. *Glob Chang Biol* **19**: 621–636.
- Elliott GN, Chou J-H, Chen W-M, Bloemberg GV, Bontemps C, Martínez-Romero E et al. (2009). *Burkholderia* spp. are the most competitive symbionts of *Mimosa*, particularly under N-limited conditions. *Environ Microbiol* **11**: 762–778.
- Ferreira AS, Silva IN, Oliveira VH, Cunha R, Moreira LM. (2011). Insights into the role of extracellular polysaccharides in *Burkholderia* adaptation to different environments. *Front Cell Infect Microbiol* **1**: 16.
- Goris J, Dejonghe W, Falsen E, De Clerck E, Geeraerts B, Willems A et al. (2002). Diversity of transconjugants that acquired plasmid pJP4 or pEMT1 after inoculation of a donor strain in the A- and B-horizon of an agricultural soil and description of *Burkholderia hospita* sp. nov. and *Burkholderia terricola* sp. nov. *Syst Appl Microbiol* **25**: 340–352.
- Groenhagen U, Baumgartner R, Bailly A, Gardiner A, Eberl L, Schulz S et al. (2013). Production of bioactive volatiles by different *Burkholderia ambifaria* strains. *J Chem Ecol* **39**: 892–906.
- Grube M, Cernava T, Soh J, Fuchs S, Aschenbrenner I, Lassek C et al. (2014). Exploring functional contexts of symbiotic sustain within lichen-associated bacteria by comparative omics. *ISME J* **9**: 412–424.
- Gutiérrez A, Caramelo L, Prieto A, Martínez MJ, Martínez AT. (1994). Anisaldehyde production and aryl-alcohol oxidase and dehydrogenase activities in ligninolytic fungi of the genus *Pleurotus*. *Appl Environ Microbiol* **60**: 1783–1788.
- Haft DH, Selengut JD, Richter RA, Harkins D, Basu MK, Beck E. (2013). TIGRFAMs and Genome Properties in 2013. *Nucleic Acids Res* **41**: D387–D395.
- Hellwig V, Grothe T, Mayer-Bartschmid A, Endermann R, Geschke FU, Henkel T et al. (2002). Altersetin, a new antibiotic from cultures of endophytic *Alternaria* spp. Taxonomy, fermentation, isolation, structure elucidation and biological activities. *J Antibiot* **55**: 881–892.
- Hondorp ER, Matthews RG. (2004). Oxidative stress inactivates cobalamin-independent methionine synthase (MetE) in *Escherichia coli*. *PLoS Biol* **2**: e336.
- Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. (2010). Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* **11**: 119.
- Kikuchi Y, Meng X-Y, Fukatsu T. (2005). Gut symbiotic bacteria of the genus *Burkholderia* in the broad-headed bugs *Riptortus clavatus* and *Leptocoris chinensis* (Heteroptera: Alydidae). *Appl Environ Microbiol* **71**: 4035–4043.
- Kilani-Feki O, Culioli G, Ortalo-Magné A, Zouari N, Blache Y, Jaoua S. (2011). Environmental *Burkholderia cepacia* Strain Cs5 acting by two analogous alkyl-quinolones and a didecyl-phthalate against a broad spectrum of phytopathogens fungi. *Curr Microbiol* **62**: 1490–1495.
- Koele N, Turpault M-P, Hildebrand EE, Uroz S, Frey-Klett P. (2009). Interactions between mycorrhizal fungi and mycorrhizosphere bacteria during mineral weathering: Budget analysis and bacterial quantification. *Soil Biol Biochem* **41**: 1935–1942.
- Ladisch MR, Lin KW, Voloch M, Tsao GT. (1983). Process considerations in the enzymatic hydrolysis of biomass. *Enzyme Microb Technol* **5**: 82–102.

- Lagesen K, Hallin P, Rodland EA, Staerfeldt HH, Rognes T, Ussery DW. (2007). RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* **35**: 3100–3108.
- Lepieux C, Turpault MP, Oger P, Frey-Klett P, Uroz S. (2012). Correlation of the abundance of betaproteobacteria on mineral surfaces with mineral weathering in forest soils. *Appl Environ Microbiol* **78**: 7114–7119.
- Lewenza S, Sokol PA. (2001). Regulation of ornibactin biosynthesis and N-Acyl-L-homoserine lactone production by CepR in *Burkholderia cepacia*. *J Bacteriol* **183**: 2212–2218.
- Lin J, Michel LO, Zhang Q. (2002). CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. *Antimicrob Agents Chemother* **46**: 2124–2131.
- Liu XY, Luo XJ, Li CX, Lai QL, Xu JH (2014). Draft genome sequence of *Burkholderia* sp. Strain MP-1, a Methyl Parathion (MP)-degrading bacterium from MP-contaminated soil. *Genome Announc* **2**: pii: e00344-14 (1–2).
- Nazir R, Boersma FGH, Warmink JA, van Elsas JD. (2010a). *Lyophyllum* sp. strain Karsten alleviates pH pressure in acid soil and enhances the survival of *Variovorax paradoxus* HB44 and other bacteria in the mycosphere. *Soil Biol Biochem* **42**: 2146–2152.
- Nazir R, Warmink JA, Boersma H, Van Elsas JD. (2010b). Mechanisms that promote bacterial fitness in fungal-affected soil microhabitats. *FEMS Microbiol Ecol* **71**: 169–185.
- Nazir R, Zhang M, de Boer W, van Elsas JD. (2012). The capacity to comigrate with *Lyophyllum* sp. strain Karsten through different soils is spread among several phylogenetic groups within the genus *Burkholderia*. *Soil Biol Biochem* **50**: 221–233.
- Nazir R, Warmink J, Voordes D, van de Bovenkamp H, van Elsas J. (2013). Inhibition of mushroom formation and induction of glycerol release—ecological strategies of *Burkholderia terrae* BS001 to create a hospitable niche at the fungus *Lyophyllum* sp. Strain Karsten. *Microb Ecol* **65**: 245–254.
- Neumann M, Mittelstadt G, Iobbi-Nivol C, Saggu M, Lendzian F, Hildebrandt P et al. (2009). A periplasmic aldehyde oxidoreductase represents the first molybdopterin cytosine dinucleotide cofactor containing molybdo-flavoenzyme from *Escherichia coli*. *FEBS J* **276**: 2762–2774.
- Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A et al. (2013). Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. *J Comput Biol* **20**: 714–737.
- Partida-Martinez LP, Hertweck C. (2007). A gene cluster encoding rhizoxin biosynthesis in “*Burkholderia rhizoxina*”, the bacterial endosymbiont of the fungus *Rhizopus microsporus*. *ChemBioChem* **8**: 41–45.
- Paul D. (2013). Osmotic stress adaptations in rhizobacteria. *J Basic Microbiol* **53**: 101–110.
- Pérez-Pantoja D, Donoso R, Agulló L, Córdova M, Seeger M, Pieper DH et al. (2012). Genomic analysis of the potential for aromatic compounds biodegradation in *Burkholderiales*. *Environ Microbiol* **14**: 1091–1117.
- Piddock LJV. (2006). Multidrug-resistance efflux pumps? not just for resistance. *Nat Rev Microbiol* **4**: 629–636.
- Pion M, Bshary R, Bindschedler S, Filippidou S, Wick LY, Job D et al. (2013). Gains of bacterial flagellar motility in a fungal world. *Appl Environ Microbiol* **79**: 6862–6867.
- Prober SM, Leff JW, Bates ST, Borer ET, Firn J, Harpole WS et al. (2015). Plant diversity predicts beta but not alpha diversity of soil microbes across grasslands worldwide. *Ecol Lett* **18**: 85–95.
- Quan CS, Zheng W, Liu Q, Ohta Y, Fan SD. (2006). Isolation and characterization of a novel *Burkholderia cepacia* with strong antifungal activity against *Rhizoctonia solani*. *Appl Microbiol Biotechnol* **72**: 1276–1284.
- Reitzer LJ, Magasanik B. (1985). Expression of *glnA* in *Escherichia coli* is regulated at tandem promoters. *Proc Natl Acad Sci USA* **82**: 1979–1983.
- Reitzer LJ, Movsas B, Magasanik B. (1989). Activation of *glnA* transcription by nitrogen regulator I (NRI)-phosphate in *Escherichia coli*: evidence for a long-range physical interaction between NRI-phosphate and RNA polymerase. *J Bacteriol* **171**: 5512–5522.
- Rice P, Longden I, Bleasby A. (2000). EMBOSS: the European Molecular Biology Open Software Suite. *Trends Genet* **16**: 276–277.
- Rivilla R, Sutton JM, Downie JA. (1995). *Rhizobium leguminosarum* NodT is related to a family of outer-membrane transport proteins that includes TolC, PrtF, CyaE and AprF. *Gene* **161**: 27–31.
- Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N, Braisted J et al. (2003). TM4: a free, open-source system for microarray data management and analysis. *Bio-Techniques* **34**: 4.
- Sass AM, Schmerk C, Agnoli K, Norville PJ, Eberl L, Valvano MA et al. (2013). The unexpected discovery of a novel low-oxygen-activated locus for the anoxic persistence of *Burkholderia cenocepacia*. *ISME J* **7**: 1568–1581.
- Schamfuss S, Neu TR, van der Meer JR, Tecon R, Harms H, Wick LY. (2013). Impact of mycelia on the accessibility of fluorene to PAH-degrading bacteria. *Environ Sci Technol* **47**: 6908–6915.
- Schattner P, Brooks AN, Lowe TM. (2005). The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. *Nucleic Acids Res* **33**: W686–W689.
- Schellenberg GD, Sarthy A, Larson AE, Backer MP, Crabb JW, Lidstrom M et al. (1984). Xylose isomerase from *Escherichia coli*. Characterization of the protein and the structural gene. *J Biol Chem* **259**: 6826–6832.
- Scherlach K, Graupner K, Hertweck C. (2013). Molecular bacteria-fungi interactions: effects on environment, food, and medicine. *Annu Rev Microbiol* **67**: 375–397.
- Schmidt S, Blom JF, Pernthaler J, Berg G, Baldwin A, Mahenthalingam E et al. (2009). Production of the antifungal compound pyrrolnitrin is quorum sensing-regulated in members of the *Burkholderia cepacia* complex. *Environ Microbiol* **11**: 1422–1437.
- Schneider T, Schmid E, de Castro JV, Cardinale M, Eberl L, Grube M et al. (2011). Structure and function of the symbiosis partners of the lung lichen (*Lobaria pulmonaria* L. Hoffm.) analyzed by metaproteomics. *Proteomics* **11**: 2752–2756.
- Schweizer H, Grussenmeyer T, Boos W. (1982). Mapping of two *ugp* genes coding for the *pho* regulon-dependent sn-glycerol-3-phosphate transport system of *Escherichia coli*. *J Bacteriol* **150**: 1164–1171.
- Sessitsch A, Coenye T, Sturz AV, Vandamme P, Barka EA, Salles JF et al. (2005). *Burkholderia phytofirmans* sp. nov., a novel plant-associated bacterium with plant-beneficial properties. *Int J Syst Evol Microbiol* **55**: 1187–1192.

- Soltani J, Hosseini Moghaddam M. (2014). Antiproliferative, antifungal, and antibacterial activities of endophytic alternaria species from Cupressaceae. *Curr Microbiol* **69**: 349–356.
- Song S, Park C. (1998). Utilization of D-ribose through D-xylose transporter. *FEMS Microbiol Lett* **163**: 255–261.
- Stopnisek N, Bodenhausen N, Frey B, Fierer N, Eberl L, Weisskopf L. (2014). Genus-wide acid tolerance accounts for the biogeographical distribution of soil Burkholderia populations. *Environ Microbiol* **16**: 1503–1512.
- Uroz S, Oger P, Morin E, Frey-Klett P. (2012). Distinct ectomycorrhizospheres share similar bacterial communities as revealed by pyrosequencing-based analysis of 16S rRNA genes. *Appl Environ Microbiol* **78**: 3020–3024.
- Vandamme P, Holmes B, Vancanneyt M, Coenye T, Hoste B, Coopman R et al. (1997). Occurrence of multiple genomovars of Burkholderia cepacia in cystic fibrosis patients and proposal of Burkholderia multivorans sp. nov. *Int J Syst Bacteriol* **47**: 1188–1200.
- Warmink JA, van Elsas JD. (2008). Selection of bacterial populations in the mycosphere of Laccaria proxima: is type III secretion involved[quest]. *ISME J* **2**: 887–900.
- Warmink JA, Nazir R, Van Elsas JD. (2009). Universal and species-specific bacterial ‘fungiphiles’ in the mycospheres of different basidiomycetous fungi. *Environ Microbiol* **11**: 300–312.
- Warmink JA, Nazir R, Corten B, van Elsas JD. (2011). Hitchhikers on the fungal highway: the helper effect for bacterial migration via fungal hyphae. *Soil Biol Biochem* **43**: 760–765.
- Williams MD, Ouyang TX, Flickinger MC. (1994). Starvation-induced expression of SspA and SspB: the effects of a null mutation in sspA on Escherichia coli protein synthesis and survival during growth and prolonged starvation. *Mol Microbiol* **11**: 1029–1043.
- Wilson K. (2001). Preparation of genomic DNA from bacteria. *Curr Protoc Mol Biol*; Chapter 2: Unit 2.4. doi:10.1002/0471142727.mb0204s56.
- Xu P, Yu H, Chakrabarty AM, Xun L (2013). Genome sequence of the 2,4,5-trichlorophenoxyacetate-degrading bacterium Burkholderia phenoliruptrix Strain AC1100. *Genome Announc* **1**: pii: e00600-13 (1–2).
- Zhang Y, Wen Z, Washburn MP, Florens L. (2010). Refinements to label free proteome quantitation: how to deal with peptides shared by multiple proteins. *Anal Chem* **82**: 2272–2281.
- Zimmer DP, Soupene E, Lee HL, Wendisch VF, Khodursky AB, Peter BJ et al. (2000). Nitrogen regulatory protein C-controlled genes of Escherichia coli: scavenging as a defense against nitrogen limitation. *Proc Natl Acad Sci USA* **97**: 14674–14679.

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