Abstract

_Pseudomonas citronellolis_ is a Gram negative, motile gammaproteobacterium belonging to the order _Pseudomonadales_ and the family _Pseudomonadaceae_. We isolated strain P3B5 from the phyllosphere of basil plants (Ocimum basilicum L.). Here we describe the physiology of this microorganism, its full genome sequence, and detailed annotation. The 6.95 Mb genome contains 6071 predicted protein coding sequences and 96 RNA coding sequences. _P. citronellolis_ has been the subject of many studies including the investigation of long-chain aliphatic compounds and terpene degradation. Plant leaves are covered by long-chain aliphates making up a waxy layer that is associated with the leaf cuticle. In addition, basil leaves are known to contain high amounts of terpenoid substances, hinting to a potential nutrient niche that might be exploited by _P. citronellolis_. Furthermore, the isolated strain exhibited resistance to several antibiotics. To evaluate the potential of this strain as source of transferable antibiotic resistance genes on raw consumed herbs we therefore investigated if those resistances are encoded on mobile genetic elements. The availability of the genome will be helpful for comparative genomics of the phylogenetically broad pseudomonads, in particular with the sequence of the _P. citronellolis_ type strain PRJDB205 not yet publicly available. The genome is discussed with respect to a phyllosphere related lifestyle, aliphate and terpenoid degradation, and antibiotic resistance.

**Keywords:** Phyllosphere, Complete genome, Pseudomonad, PacBio, Nonhybrid de novo assembly, Alkane degradation, Terpenoid degradation, Bioremediation potential

Introduction

The genus _Pseudomonas_ encompasses a large group of bacteria that are ecologically and functionally very diverse including many human and plant pathogenic species [1], but also species with mutualistic host interactions, the most prominent examples of which are biocontrol strains that protect plants from pathogens [2–4]. Due to their versatile properties and pathogenicity, pseudomonads garnered much attention in recent years and members of the genus have been the subject of full genome sequencing projects, i.e. at the time of writing the _Pseudomonas_ Genome Database contained 98 complete genomes and 1447 draft genomes [5]. Up till now, this database is heavily biased towards pathogenic species, most prominently including 996 human-pathogenic _P. aeruginosa_ strains and 105 plant pathogenic _P. syringae_ strains. Taking into account the assigned species of the sequenced strains only, roughly a third (76 of 199) of the validly published _Pseudomonas_ species [6] have been sequenced. Thus, a comprehensive picture of genetic diversity within the pseudomonads is lacking and investigating thus far unsequenced species will fill this knowledge gap.
**Pseudomonas citronellolis** has long been recognized for its ability to degrade hydrocarbons such as isoprenoid compounds including citronellol, hence its name [7], and complex oily sludge contaminations [8], indicating the species might potentially serve as bioremediation organism by cleaning up oil contaminated sites. The described strain was isolated from plant leaf material. Interestingly, plant leaves are covered by a cuticle consisting of cutin, an esterified aliphatic polymer [9] that is impregnated and overlaid by intra- and epi-cuticular waxes consisting of very long-chain aliphatic compounds, including alkanes, alcohols, and fatty acids [10]. The ability of *P. citronellolis* to degrade aliphatic compounds leads to the intriguing possibility that the bacterium may be able to exploit aliphatic compounds as a nutrient source during leaf surface colonization. Furthermore, the ability of *P. citronellolis* to also degrade terpenoid compounds [11] is another interesting feature that might enable this species to degrade the abundantly available terpene compounds from herbal plants [12] and hence may represent a growth advantage over other phyllosphere bacteria on such hosts.

*P. citronellolis* has previously been used as a model organism to investigate metabolic pathways and enzyme activity, such as glucose catabolism and gluco-neogenesis [13], acyl-coenzyme A carboxylases [14], and terpene degradation [11]. The complete genome of *P. citronellolis* described here represents a useful resource for the ongoing environmental and physiological research in this species, and can serve as a starting point for detailed systems biology studies [15], at least until the genome sequence of the type strain PRJDB205 becomes available. We present a summary, classification and general physiological features of the strain *P. citronellolis* P3B5, as well as the genomic sequence, assembly, annotation, and its putative adaptations to a phyllosphere lifestyle.

**Organism information**

**Classification and features**

The species *P. citronellolis* was proposed and described by Seubert [7] and isolated from soil collected under pine trees in northern Virginia, USA. *P. citronellolis* is a Gram negative, rod-shaped, gammaproteobacterium that is approximately 2 μm in length and 0.5 μm in width (Fig. 1a), motile by one polar flagellum, and non-spore-forming [7]. On Lysogeny Broth agar *P. citronellolis* forms white, round colonies (Fig. 1b), that produce green fluorescent pigments after several days of incubation. Grown in liquid M9 minimal medium the production of green fluorescent pigments is even more pronounced (Fig. 1c).

![Fig. 1 a Scanning-electron micrograph of *P. citronellolis* P3B5. b *P. citronellolis* P3B5 grown on LB agar for 4 days. c *P. citronellolis* P3B5 grown in M9 minimal medium for 20 h excited by UV light exhibiting strong fluorescence. d Growth of *P. citronellolis* P3B5 was analyzed by measuring the optical density at 600 nm at the different temperatures for 24 h. 12 to 15 replicate measurements were performed for each temperature. By plotting the observed growth rate during the exponential growth phase at different temperatures, it was determined that the ideal growth temperature of *P. citronellolis* P3B5 is around 37 °C. No growth was observed at 4 °C.](image)

The here-described *P. citronellolis* P3B5 was recovered from healthy basil leaves. The species was initially identified by MALDI biotyping using a MicroFlex MALDI-TOF mass spectrometer, and the MALDI Biotyper DB V4.0.0.1 (Bruker Daltonics, Germany). MALDI biotyping has been shown to be able to identify pseudomonads at the species level with high accuracy [16, 17].

To isolate bacteria from the basil phyllosphere, 30 g of plant material were suspended in 100 ml peptone water (9 g/L NaCl, 1 g/L tryptone (Merck, Germany)), processed for 3 min in a stomacher (Smasher, AES Chemunex, France) and 100 μL aliquots of the resulting supernatant were plated onto different media, including the *Escherichia coli* selective TBX agar (Oxoid, UK). *P. citronellolis* P3B5 was isolated from the background microbiota on TBX agar, i.e. non-target bacteria growing on the agar, indicating that *P. citronellolis* utilizes similar compounds as *E. coli*. Furthermore, since the incubation took place at 44 °C, it is able to grow at high temperatures. However, the characteristic blue color indicative for *E. coli* colonies on TBX agar was not formed by *P. citronellolis*, indicating the absence of a functional β-glucuronidase in its genome.

During the survey not only *P. citronellolis* P3B5, but additional, not further characterized *P. citronellolis* isolates were frequently detected in marjoram and basil phyllosphere samples, implying *P. citronellolis* to be part...
of the plants indigenous phyllosphere communities. Pseudomonads are common colonizers of the phyllosphere [18] and can contribute to large proportions of its bacterial community [19–21].

To phylogenetically place P. citronellolis P3B5, a phylogenetic tree was constructed by multiple genome alignment using the program progressiveMauve with standard parameters [22] (Fig. 2). From each thus far sequenced species one representative strain, if possible a type strain, was chosen. Only complete genomes were considered. As outgroup species Xanthomonas campes- tris pv. campestris ATCC 33913 was chosen. A summary of the classification and general features of P. citronelle- lis P3B5 is given in Table 1.

**Biochemical profiling**

To perform a detailed biochemical characterization of P. citronellolis P3B5, it was cultivated overnight in 25 mL MM2 medium containing L-asparagin and sorbitol (4 g l\(^{-1}\) L-asparagine, 2 g l\(^{-1}\) K\(_2\)HPO\(_4\), 0.2 g l\(^{-1}\) MgSO\(_4\), 3 g l\(^{-1}\) NaCl, 10 g l\(^{-1}\) sorbitol) at 28 °C and 240 rpm, before it was harvested by centrifugation at 3500 \(\times\) g for 10 min. The harvested cells were washed thrice in 1 \(\times\) PBS buffer (8 g l\(^{-1}\) NaCl, 0.2 g l\(^{-1}\) KCl, 1.44 g l\(^{-1}\) Na\(_2\)HPO\(_4\), 0.24 g l\(^{-1}\) KH\(_2\)PO\(_4\), pH 7), before they were resuspended and diluted in 1 \(\times\) PBS to reach an optical density of OD\(_{600\text{nm}}\) = 0.1. This suspension was then used for inoculation of Biolog GN2, GenIII, and AN plates (Biolog Inc, USA), which were incubated for several days at 28 °C and analyzed for changes of their optical density at 590 nm using a microtiter plate reader (Infinite M200, Tecan, Switzerland).

Analysis of the GN2 plates revealed that P. citronellolis P3B5 was able to utilize the following wide range of substrates: 2-aminoethanol, α-ketoglutaric acid, α-d-glucose, β-hydroxy butyric acid, bromo succinic acid, cis-aconitic acid, citric acid, d-alanine, d-cellobiose, d-fructose, d-galactonic acid lactone, d-galacturonic acid, d-gluconic acid, d-mannitol, d-mannose, d-melibiose, d-raffinose, d,l-α-glycerol phosphate, d,l-lactic acid, γ-amino butyric acid, glucose-1-phosphate, glucose-6-phosphate, hydroxy-l-proline, inosine, itaconic acid, l-alanine, l-arabinose, l-asparagine, l-aspartic acid, l-glutamic acid, l-histidine, l-proline, l-pyroglutamic acid, methyl pyruvate, mono-methyl-succinate, p-hydroxy phenylacetic acid.
propionic acid, putrescine, quinic acid, sebacic acid, succinic acid, succinic acid, sucrose, tween 40, tween 80, and urocanic acid. On Biolog GenIII plates, the following additional substrates were utilized: Acetic acid, α-hydroxybutyric acid, α-ketobutyric acid, d-fructose-6-phosphate, d-galactose, d-glucose-6-phosphate, d-glucuronic acid, d-malic acid, d-maltose, d-trehalose, formic acid, l-arginine, l-galactonic acid lactone, l-lactic acid, l-malic acid, N-acetyl-d-neuramic acid, and pectin.

In contrast to previously described *P. citronellolis* strains, *P. citronellolis* P3B5 could not utilize d-mannitol, glycerol, d,l-carnitine, d-psicose, l-alanyl-glycine, and formic acid. However, compared to the previously described *P. citronellolis* strains, *P. citronellolis* P3B5 additionally utilized α-d-lactose, d-galactose, d-glucose-6-phosphate, d-glucuronic acid, d-maltose, d-mannitol, d-melibiose, d-raffinose, d-saccharic acid, d-trehalose, d,l-α-glycerol phosphate, glucose-1-phosphate, glucose-6-phosphate, hydroxy-l-proline, inosine, l-arabinose, l-galactonic acid lactone, N-acetyl-d-glucosamine, succrose, and thymidine [7, 23]. The following compounds, that have not been tested in previous studies, were also utilized by *P. citronellolis* P3B5: α-hydroxy-butyric acid, α-ketobutyric acid, d-fructose-6-phosphate, d-mannose, fumaric acid, l-alanyl-l-histidine, l-alanyl-l-glutamine, l-glutamine, l-lactic acid, l-pyroglutamic acid, l-rhamnose, l-valine plus l-aspartic acid, N-acetyl-d-neuramic acid, p-hydroxy phenylacetic acid, pyruvic acid, quinic acid, and succinamic acid. In summary, based on the tested set of substrates, *P. citronellolis* P3B5 appears to be metabolically more versatile than previously isolated strains, which might reflect an adaptation to the phyllosphere environment.

### Table 1 Classification and general features of *P. citronellolis* P3B5 [30]

| MIGS ID | Property                | Term                                      | Evidence code |
|---------|-------------------------|-------------------------------------------|---------------|
|         | Classification          | Domain Bacteria                           | TAS [62]      |
|         |                         | Phylum Proteobacteria                     | TAS [63]      |
|         |                         | Class Gammaproteobacteria                 | TAS [64]      |
|         |                         | Order Pseudomonadales                     | TAS [65, 66]  |
|         |                         | Family Pseudonosadaee                     | TAS [66, 67]  |
|         |                         | Genus Pseudomonas                          | TAS [66, 68]  |
|         |                         | Species Pseudomonas citronellolis         | TAS [7, 23, 66] |
|         | Gram stain              | Negative                                  | TAS [7]       |
|         | Cell shape              | Rod                                       | TAS [7]       |
|         | Motility                | Motile                                    | TAS [7]       |
|         | Sporulation             | Not reported                              | TAS [7]       |
|         | Temperature range       | 18–42 °C                                  | IDA           |
|         | Optimum temperature     | 37 °C                                     | IDA           |
|         | pH range; Optimum       | not determined                            | IDA           |
|         | Carbon source           | See paragraph “biochemical profiling”     | IDA           |
| MIGS-6  | Habitat                 | Soil, phyllosphere                        | TAS [7], IDA  |
| MIGS-6.3| Salinity                | 1–5 % NaCl (w/v)                          | IDA           |
| MIGS-22 | Oxygen requirement      | Aerobic                                   | TAS [7]       |
| MIGS-15 | Biotic relationship     | Free living, symbiont                     | TAS [7], IDA  |
| MIGS-14 | Pathogenicity           | Non-pathogen                              | NAS           |
| MIGS-4  | Geographic location     | Switzerland/Zurich area                   | IDA           |
| MIGS-5  | Sample collection       | 02.07.2015                                | IDA           |
| MIGS-4.1| Latitude                | 47°45’37 N                                | IDA           |
| MIGS-4.2| Longitude               | 8°43’7 E                                  | IDA           |
| MIGS-4.4| Altitude                | 521 m                                     | IDA           |

* Evidence codes--IDA Inferred from Direct Assay, TAS Traceable Author Statement (i.e., a direct report exists in the literature), NAS Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [69].

**Growth of *P. citronellolis* P3B5 at different temperatures**

*P. citronellolis* was incubated in 12 to 15 replicates each at 44, 42, 37, 33, 28, 20, and 16 °C in tryptic soy broth
Resilience to abiotic factors and antibiotic resistance

On Biolog GenIII plates, *P. citronellolis* P3B5 was able to grow to the same optical density as the positive control in 2 % NaCl solution, to a lower optical density in 5 % NaCl solution, and was unable to grow in 9 % NaCl solution. It was not inhibited by 1 % sodium lactate, rifamycin SV, minocycline, lincomycin, niaprof 4, vancomycin, nalidixic acid, potassium tellurite, and aztreonam. Growth, however not to the same optical density as the positive control, was observed in the presence of fusidic acid, troleandomycin, guanidine HCl, and sodium bromate. No significant growth was detected in presence of d-serine, lithium chloride, and sodium butyrate.

Pseudomonads were previously described to be key players in propagating plasmids, including ABR bearing plasmids, in the phyllosphere [24–28]. Therefore, additional ABR exhibited by *P. citronellolis* P3B5 were determined in antibiotic disc diffusion assays [29]. Out of the tested 32 clinically relevant antibiotics or antibiotic combinations, *P. citronellolis* P3B5 was resistant against ten antibiotics or combinations including the β-lactams cefoxitin, cefpodoxime, cefuroxime, temocillin, cephalothin, cefotaxime, the β-lactam/β-lactamase inhibitor mix amoxicillin/clavulanic acid, and several antibiotics of other classes, i.e. trimethoprim, trimethoprim/sulfamethoxazole, nitrofurantoin, and fosfomycin. *P. citronellolis* P3B5 was not resistant against the following tested antibiotics: cefepime, colistin, tobramycin, gentamycin, amikacin, ciprofloxacine, levofloxacine, sulfonamide, imipenem, and ceftazidime. Resistance was defined based on existing cutoffs, or, when no cutoff was available, as grown completely up to the antibiotic containing disc [29].

Genome sequencing information

Genome project history

The organism was selected for sequencing as part of an ongoing project investigating the bacterial diversity on the plant surface (i.e., the phyllobiome) of basil (*Ocimum basilicum* L.). The sequencing project was completed in December 2015; the sequencing data was deposited as a complete genome (one contig representing the complete genome of *P. citronellolis* P3B5) in Genbank under BioProject PRJNA309370, with the accession number CP014158. The genome was sequenced with the Pacific Biosciences RS II platform (Microsynth AG, Switzerland). A summary of the project according to the MIGS version 2.0 [30] is given in Table 2.

Growth conditions and genomic DNA preparation

To isolate gDNA, cells were grown overnight in terrific broth (12 g l⁻¹ tryptone, 24 g l⁻¹ yeast extract, 4 ml l⁻¹ glycerol, 100 ml l⁻¹ 0.17 M KH₂PO₄, 0.72 M K₂HPO₄) at 30 °C and 250 rpm. gDNA was extracted using the GenElute Bacterial DNA Kit (Sigma-Aldrich, USA) according to the manufacturer's recommendations. To concentrate gDNA, the primary eluate was precipitated using 5 M ammonium acetate and 100 % ethanol followed by a washing step using 70 % ethanol. The quality and quantity of the extracted DNA was evaluated on a 1.0 % (w/v) agarose gel, by measuring absorption ratios 260 nm/280 nm, and 260 nm/230 nm, and additionally by performing a Qubit dsDNA GR assay (Life Technologies, USA). To determine the presence of plasmids, a plasmid extraction using the NucleoSpin Plasmid kit (Macherey-Nagel, Germany) was performed according to the manufacturer’s recommendations. However, no plasmids could be detected after running the sample on a 1.0 % (w/v) agarose gel.

| MIGS ID | Property | Term |
|---------|----------|------|
| MIGS 31 | Finishing quality | Complete |
| MIGS-28 | Libraries used | 20 Kb PacBio library (BluePippin size selection) |
| MIGS 29 | Sequencing platforms | PacBio RS II |
| MIGS 31.2 | Fold coverage | 148x |
| MIGS 30 | Assemblers | HGAP.3 |
| MIGS 32 | Gene calling method | Prodigal 2.60 |
| Locus Tag | | PcP3B5 |
| Genbank ID | | CP014158 |
| GenBank Date of Release | | 10.03.2016 |
| BIOPROJECT | | PRJNA309370 |
| MIGS 13 | Source Material Identifier | P3B5 |
| Project relevance | | phyllosphere, environmental, biochemistry, and bioremediation |
Figure S1). Subsequent de novo genome assembly and resequencing steps were performed using PacBio SMRT Portal 2.3.0 [31]. The assembly was generated using the PacBio SMRT Portal protocol RS_HGAP_Assembly.3. Terminal repeats were removed, the genome circularized and its start position was aligned with the dnaA gene using Circulator 1.1.2 [32]. Several rounds of sequence polishing using PacBio SMRT Portal protocol RS_Resequencing.1 with stringent filter criteria (“Minimum Polymerase Read Quality”: 84) were performed, resulting in one 6,951,444-bp contig with an average coverage depth of 148-fold. The assembly did not contain any plasmids and, since virtually all reads were mapped to the genome (97.1 %, other reads were of low quality or chimeric and could still be attributed to the chromosome after manual inspection), provided no evidence for the existence of plasmids. This is in line with the results of the agarose gel electrophoresis analysis described above.

**Genome annotation**

Genome annotation was performed using Prokka 1.11.0 [33] with ncRNA search enabled and incorporation of SignalP 4.1 to predict signal peptide cleavage sites [34]. The annotation of selected ORFs annotated as “misc_rRNA” by Prokka was manually curated to conform to NCBI requirements. Predicted CDS were then searched against Pfam-A v27.0 [35] and TMHMM 2.0c [36] databases using InterProScan 5.13 [37]. COG categorization was performed by searching predicted CDS against the EggNOG 4.5 database using the Gammaproteobacteria specific dataset [38] and subsequent extraction of COG categories.

**Genome properties**

The properties of the complete genome sequence of *P. citronellolis* P3B5 are summarized in Table 3. The average GC content was 67.11 %. Of the 6169 predicted genes, 6071 (98.41 %) were protein CDS of which 4762 genes had a function prediction. Genes without functional prediction by Prokka were annotated as “hypothetical protein”. Two pseudo genes (PcP3B5_29180 and PcP3B5_42810) were predicted by the NCBI annotation pipeline [39] and then incorporated into the Prokka annotation. Of the predicted protein coding genes, 5523 were assigned to COGs of 22 classes (Table 4, Fig. 3). Using the Pfam database, 5242 CDS were assigned to a protein family. Putative transmembrane domains were predicted for 1263 CDS. A signal peptide was predicted for 702 CDS. A total of 96 RNA genes were predicted including 15 rRNA (five complete rRNA operons each comprising a 23S, 16S and 5S rRNA gene), 76 tRNA, 1 tmRNA, and 4 ncRNA genes. Binding sites for cobalamin and thiamine pyrophosphate were predicted by the NCBI annotation pipeline. One CRISPR repeat was predicted by Prokka and further confirmed by CRISPRFinder [40] and PILER-CR [41]. However, no evidence for a Cas protein-coding gene was found in the genome. An analysis of putative prophages using PHAST [42] resulted in six hits in the genome, three of which were designated as intact prophages (PcP3B5_02970–PcP3B5_03410; PcP3B5_06890–PcP3B5_07120; PcP3B5_45940–PcP3B5_46520) and the other three as incomplete prophages (PcP3B5_40450–PcP3B5_40640; PcP3B5_40870–PcP3B5_41140; PcP3B5_46670–PcP3B5_46960). Genomic islands and ABR genes within genomic islands were predicted using IslandViewer 3 [43]. As references for genomic island analysis, the closely related genomes of *P. knackmussii* B13, *P. denitrificans* ATCC 13867, *P. aeruginosa* PAO1, and *P. stutzeri* DSM 4166 were chosen.

**Extended insights from the genome sequence**

The *P. citronellolis* P3B5 genome in the light of a phyllosphere associated lifestyle

Plant leaf surfaces, often referred to as phyllosphere, represent an extreme environment to its colonizers which are exposed to largely fluctuating levels of drought, DNA-damaging UV radiation, heat, and oligotrophic nutrient conditions [18]. At the micrometer scale the phyllosphere is very heterogeneous, i.e. habitable sites and nutrient availability is discontinuous, tremendously impacting its colonizers [44, 45].

**Resilience to phyllosphere stress factors**

*P. citronellolis* P3B5 did not only survive, but grew at temperatures as high as 44 °C and was also able to grow at moderate temperatures of 16 °C. This ability might represent a fitness advantage on plant leaf surfaces that often feature fluctuating and high temperatures [18].

| Table 3 Genome statistics | Value | % of Total |
|---------------------------|-------|------------|
| Genome size (bp)          | 6951444| 100.00     |
| DNA coding (bp)           | 6028113| 86.72      |
| DNA G+C (bp)              | 4665300| 67.11      |
| DNA scaffolds              | 1      | 100.00     |
| Total genes               | 6169   | 100.00     |
| Protein coding genes      | 6071   | 98.41      |
| RNA genes                 | 96     | 1.56       |
| Pseudo genes              | 2      | 0.03       |
| Genes in internal clusters| NA     | NA         |
| Genes with function prediction | 4762 | 77.19     |
| Genes assigned to COGs    | 5523   | 89.53      |
| Genes with Pfam domains   | 5242   | 84.97      |
| Genes with signal peptides| 702    | 11.38      |
| Genes with transmembrane helices | 1263 | 20.47 |
| CRISPR repeats            | 1      | 1          |
Adaptation to fluctuating and high temperatures is also reflected in the genome which encodes several heat shock proteins including DnaK (PcP3B5_54370, PcP3B5_56190), GroEL (PcP3B5_12480), and the cold shock proteins CspA_1-CspA_4 (PcP3B5_06040, PcP3B5_17140, PcP3B5_45760, PcP3B5_47880). To counter oxidative stress, *P. citronellolis* P3B5 is equipped with genes encoding proteins known to be involved in oxidative stress reduction such as a manganese-based superoxide dismutase (PcP3B5_11610), a ferrous-based superoxide dismutase (PcP3B5_52370), four catalases (PcP3B5_07860, PcP3B5_23220, PcP3B5_27040, PcP3B5_31600), and several peroxidases (PcP3B5_10150, PcP3B5_32470, PcP3B5_44760). To counter drought stress, *P. citronellolis* P3B5 is equipped with genes encoding for the production of trehalose (PcP3B5_27330) and uptake or production of betaine (PcP3B5_00410–00420, PcP3B5_01390, PcP3B5_01330–01360, PcP3B5_17690, PcP3B5_02060–02090, PcP3B5_25880, PcP3B5_26750–26770, PcP3B5_28710–28740, PcP3B5_28870, PcP3B5_29850, PcP3B5_31730–31770, PcP3B5_34470–34500, PcP3B5_34590, PcP3B5_36730, PcP3B5_43400, PcP3B5_45400, PcP3B5_48640, PcP3B5_50450, PcP3B5_58450–58430, PcP3B5_60970, PcP3B5_61100, PcP3B5_61120–61140, PcP3B5_61750, PcP3B5_62040), two osmoprotectants. Notably, no other representative pseudomonad currently found in the SEED database [46] features as many betaine related uptake systems, indicating the importance of this osmoprotectant for the lifestyle of *P. citronellolis* P3B5. This suggests a phyllosphere adapted lifestyle which requires the ability to survive and thrive under constant drought stress. The phyllosphere has furthermore been described to be iron limited [47], therefore, the acquisition of and competition for iron is crucial on plant leaves. The *P. citronellolis* P3B5 genome, as is common in pseudomonads, encodes for several genes involved in the acquisition of iron by the production and uptake of siderophores.

**Potential resource utilization on plant surfaces**

*P. citronellolis* P3B5 is equipped for the acquisition and utilization of many nutrients that are available in the phyllosphere, especially different carbohydrates and amino acids [48, 49]. Genes encoding for ABC transporters for amino acids found on plant surfaces

| Code | Value | % age | Description |
|------|-------|-------|-------------|
| J    | 189   | 3.11  | Translation, ribosomal structure and biogenesis |
| A    | 1     | 0.02  | RNA processing and modification |
| K    | 480   | 7.91  | Transcription |
| L    | 180   | 2.96  | Replication, recombination and repair |
| B    | 5     | 0.08  | Chromatin structure and dynamics |
| D    | 40    | 0.66  | Cell cycle control, Cell division, chromosome partitioning |
| V    | 75    | 1.24  | Defense mechanisms |
| T    | 286   | 4.71  | Signal transduction mechanisms |
| M    | 281   | 4.63  | Cell wall/membrane biogenesis |
| N    | 114   | 1.88  | Cell motility |
| U    | 79    | 1.30  | Intracellular trafficking and secretion |
| O    | 182   | 3.00  | Posttranslational modification, protein turnover, chaperones |
| C    | 417   | 6.87  | Energy production and conversion |
| G    | 196   | 3.23  | Carbohydrate transport and metabolism |
| E    | 489   | 8.05  | Amino acid transport and metabolism |
| F    | 112   | 1.84  | Nucleotide transport and metabolism |
| H    | 160   | 2.64  | Coenzyme transport and metabolism |
| I    | 218   | 3.59  | Lipid transport and metabolism |
| P    | 349   | 5.74  | Inorganic ion transport and metabolism |
| Q    | 142   | 2.34  | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 0     | 0.00  | General function prediction only |
| S    | 1528  | 25.17 | Function unknown |
|     | 548   | 9.03  | Not in COGs |

The total is based on the total number of protein coding genes in the genome.
(arginine, cysteine, glycine, histidine, methionine, proline), dipeptides, oligopeptides, branched amino acids, putrescine, spermidine, lipopolysaccharides, glucose, and nitrate. Furthermore, genes encoding for a phosphotransferase uptake system for fructose are present, as well as major facilitator superfamily protein sugar transporter and other sugar transporters. To predict the ability of \textit{P. citronellolis} P3B5 to metabolize various compounds, RAST [50] and modelSEED were employed (http://modelseed.org) [46, 51, 52]. Genetic evidence for the metabolic utilization of glucose, fructose, and sucrose, the three most prevalent photosynthates found on leaves, was found [48, 53]. Furthermore, genes predicted to be involved in the degradation of short organic acids which can be found on plant leaves were detected, e.g. citrate, fumarate, glycolate, malate, maleate, pyruvate, succinate, and tartrate [48].

Recently, the soil-borne \textit{Pseudomonas aeruginosa} SJTD-1, a strain phylogenetically related to \textit{P. citronellolis}, was shown to be able to degrade medium and long chain alkanes from n-tetradecane (C_{14}) to n-tetracosane (C_{24}) due to the protein alkane monooxygenase [54]. The presence of two AlkB-like alkane monooxygenase genes in the \textit{P. citronellolis} P3B5 genome (PcP3B5\_23990, PcP3B5\_31900) might enable it to nutritionally access long and very-long chain alkanes available on plant leaves, thereby gaining a fitness advantage over other phyllosphere colonizers. Furthermore, \textit{P. citronellolis} P3B5 contains two putative AlmA-like monooxygenases (PcP3B5\_03500, PcP3B5\_37240), which would
allow the degradation of very long chain alkanes (>C₃₀) [55]. *P. citronellolis* P3B5 was isolated from herbs that are rich in aromatic oils, such as terpenes [12]. Previously it has been shown that *P. citronellolis* is able to degrade terpenes such as citronellol [11] and a corresponding gene cluster (PcP3B5_19950–20010) was also detected in *P. citronellolis* P3B5, which may be responsible for enabling this species to colonize the phyllosphere of aromatic herbs.

**Biotic relationship to the plant host**

*P. citronellolis* P3B5 and other *P. citronellolis* isolates were isolated from healthy plant material and plants from the same field plot did not exhibit disease symptoms during the course of the survey. Therefore, it is unlikely that *P. citronellolis* is a plant pathogen. It is unclear if the strain is able to confer a health promoting effect on its plant host, thus it is prudent to classify it as tritagonist, an organism whose biotic relationship to its host is thus far undescribed [56]. However, the *P. citronellolis* P3B5 genome contains genes whose products are involved in the biosynthesis of indole-3-acetic acid (PcP3B5_05210–05220, PcP3B5_17810, PcP3B5_07120–07140), a compound often found to be produced by bacteria associated with plants [18]. Indole-3-acetic acid is a plant hormone of the auxin class which induces plant cell elongation and division, which leads to an increase of local available nutrients [57]. This indicates that *P. citronellolis* P3B5 is able to impact on the plant host.

**Antibiotic resistance and possible horizontal gene transfer of resistance genes**

The genomic data broadly supports the phenomenological antibiotic screens described above, the respective putative ABR genes and loci are summarized in Table 5. Many of the tested compounds were β-lactam antibiotics of different generations. The P3B5 genome contains genes encoding for six predicted β-lactamases, providing resistances against many β-lactam antibiotics, including a class C β-lactamase conferring resistance to cephalosporin antibiotics and putatively to the monobactam aztreonam. Furthermore, the genome contains genes encoding for efflux pumps that provide resistances to other antibiotics, such as trimethoprim.

To further analyze the potential of described ABR genes to be mobilized by horizontal gene transfer, it was investigated if they are located on mobile genetic elements, i.e. prophages, transposons, and genomic islands. None of the six putative prophage related genomic loci or the predicted genomic islands contained genes encoding for ABR. Furthermore, after close inspection of flanking regions 30 kbp up- and downstream of resistance genes, no evidence for functional transposable elements could be detected.

| Antibiotic | Class | Encoded resistance genes; locus |
|------------|-------|---------------------------------|
| amoxicillin/clavulanic acid | β-lactam + β-lactamase inhibitor |  |
| cefotaxime | β-lactam/third generation cephalosporin |  |
| cefoxitin | β-lactam/second generation cephalosporin |  |
| cefpodoxime | β-lactam/third generation cephalosporin |  |
| cefuroxime | β-lactam/second generation cephalosporin |  |
| cephalothin | β-lactam/second generation cephalosporin | metallo-β-lactamase, PcP3B5_32180 class A β-lactamase, PcP3B5_44400 metallo-β-lactamase, PcP3B5_46940 metallo-β-lactamase, PcP3B5_47300 |
| cefuroxime | β-lactam/third generation cephalosporin |  |
| cefuroxime | β-lactam/third generation cephalosporin |  |
| lincosamide |  | intrinsic resistance |
| vancomycin |  | intrinsic resistance |
| minocycline | tetracycline antibiotic | tetA; PcP3B5_30660 |
| rifamycin SV | rifamycin |  |
| trimethoprim | sulfonamide antibiotic |  |
| trimethoprim/ sulfamethoxazole | dihydropyrimidinase reductase inhibitor/ sulfonamide antibiotic |  |
| nitrofurantoin | nitrofuran derivative | intrinsic resistance and vanX; PcP3B5_55530 |
| fosfomycin | N-acetylmuramic acid synthesis inhibition | fosA; PcP3B5_27860 |
Bioremediation potential
The capability of *P. citronellolis* P3B5 to degrade long-chain alkanes and terpenes in combination with a predicted resilience to many environmental stresses make it an ideal candidate for future bioremediation applications. Also, a recent study highlighted the ability of a *P. citronellolis* isolate to be able to degrade low density polyethylene [58] and, moreover, *P. citronellolis* P3B5 is closely related to *P. knackmussi*, which is known for its potential in biodegradation of chloroaromatic compounds [59]. This further hints towards a, yet to be further investigated, broad biodegradation potential of *P. citronellolis*. Its preferred niche on plant surfaces can potentially be further exploited for so-called “phylllo-remediation” approaches during which bacteria are employed to degrade organic-pollutants on plant foliage [60, 61].

Conclusions
We provide a comprehensive insight into the genome and biochemical properties of the environmentally and biotechnologically interesting species *P. citronellolis*. Furthermore, we provide an interpretation of its environmental life-style on plant leaves from a genomic perspective. The *P. citronellolis* P3B5 genome encodes genes that should enable it to degrade long- and very long-chain alkanes, and terpenes. This metabolic capability, in combination with its stress-resilience and phyllosphere lifestyle, makes the organism an intriguing candidate for phyllo-remediation based bioremediation approaches. Resistance to several ABs was observed and several ABR genes were detected, but no evidence for the potential of ABR gene mobilization could be found. The availability of the complete genome sequence of *P. citronellolis* will facilitate future comparative genomics approaches of the phylogenetically broad genus *Pseudomonas*, which is currently understudied and biased towards pathogenic species. To fully appreciate the genetic diversity of the genus even more species should be complete genome sequenced in the future. This will also enable hypothesis-driven research on the difference between pathogenic and non-pathogenic species.

Additional files

Additional file 1: Table S1. Accession numbers of representative *Pseudomonas* genomes used to generate the phylogenetical trees in Fig. 2 and Additional file 2: Figure S2. (DOCX 70 kb)

Additional file 2: Figure S1. Subread length distribution after sequencing. Figure S2. Phylogenetic tree of the genus *Pseudomonas* highlighting the position of *P. citronellolis* P3B5 relative to other representative *Pseudomonas* species. *Xanthomonas campestris pv. campestris* ATCC 33913 was chosen as outgroup. The tree is based on a MLSA using four housekeeping gene sequences (16S rRNA, gyrB, rpoB, rpoD). The bar represents the number of base substitutions per site. The percentage of replicate trees in which associated taxa clustered in the bootstrap test with 1000 replicates are shown next to the respective branches. Accession numbers of the used strains are reported in Additional file 1: Table S1. (DOCX 295 kb)

Abbreviations
ABR: Antibiotic resistance; gDNA: genomic DNA; MALDI: Matrix-assisted laser desorption/ionization; MALDI-TOF: MALDI-time of flight; MLSA: Multilocus sequence analysis; TBX: agar: Tryptone Bile X-Glucuronide agar

Acknowledgments
The authors thank Diane Gossin for help with the isolation of the strain and Adithi Ravikumar Varadarajan for constructive discussions on genome annotation and phylogenetic analyses. The authors acknowledge support of the Scientific Center for Optical and Electron Microscopy ScopeM of the Swiss Federal Institute of Technology ETHZ and thank Anne Greet Bittermann (ScopeM) for her excellent help with electron microscopy. Reinhard Zbinden is acknowledged for his help and supervision during antibiotic resistance assays. Ulrich Zürcher is acknowledged for the management of the research program REDYMO. This work was financially supported by the Agroscope research programs REDYMO and MicBioDiv.

Author contributions
MRE conceived and planned the experimental work, performed the biochemical and growth characterization, isolated DNA, analyzed data and the genome, and wrote the manuscript. MS planned and performed the bioinformatics analysis, assembled the genome, annotated the genome, performed phylogenetic analysis, analyzed data, and contributed to the writing of the manuscript. M-TG performed and analyzed the antibiotic resistance screen. CP performed and analyzed the biochemical and growth characterization. JEF planned the bioinformatics analysis. CHA planned and supervised the bioinformatics analysis, annotated the genome, and wrote the manuscript. OD conceived the study and wrote the manuscript. All authors critically read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Author details
1Agroscope, Institute for Food Sciences IFS, Wädenswil, Switzerland. 2Agroscope, Institute for Plant Production Sciences IPS, Wädenswil, Switzerland. 3Swiss Institute of Bioinformatics, Wädenswil, Switzerland. 4ETH Zurich, Institute of Food, Nutrition and Health, Zurich, Switzerland.

Received: 12 March 2016 Accepted: 31 August 2016
Published online: 26 September 2016

References
1. Peix A, Ramírez-Bahena M-H, Velázquez E. Historical evolution and current status of the taxonomy of genus Pseudomonas. Infect Genet Evol. 2009;9:1132–47.
2. Loper IE, Kobayashi DY, Paulsen IT. The Genomic Sequence of Pseudomonas fluorescens Pf-5: Insights Into Biological Control. Phytopathology. 2007;97:233–8.
3. DeVries M, Pandey P, Bucheli TD, Varadarajan AR, Ahrens CH, Weisskopf L, Bailly A. Volatile organic compounds from native potato-associated *Pseudomonas* as potential anti-oomycete agents. Front Microbiol. 2015; 6:1295.
4. Ramette A, Frapelli M, Saux MF-L, Grufza C, Meyer J-M, Défago G, Sutra L, Moinne-Locouz Y. Pseudomonas protegens sp. nov., widespread plant-protecting bacteria producing the biocontrol compounds 2,4-diacetylphloroglucinol and pyoluteorin. Syst Appl Microbiol. 2011;34: 180–8.
5. Winsor GL, Van Rossum T, Lo R, Kiliaan B, Whiteside MD, Hancock RE, Brinkman FS. Pseudomonas Genome Database: facilitating user-friendly, comprehensive comparisons of microbial genomes. Nucleic Acids Res. 2009;37:D483–8.
6. Taxon Abstract for the genus *Pseudomonas*. NamesforLife, LLC. http://doi.org/10.1601/tx.2552. Accessed 28 Aug 2016.
7. Seubert W. DEGRADATION OF ISOPRENOID COMPOUNDS BY MICROORGANISMS. I, Pseudomonas citronellolis n. sp: Isolation and Characterization of an Isoprenoid-Degrading Bacterium. J Bacteriol. 1960;79:426–34.
8. Bhattacharya D, Sarma PM, Krishnan S, Mishra S, Bal B. Evaluation of Genetic Diversity among Pseudomonas citrinovirogenes Strains Isolated from Oily Sludge-Contaminated Sites. Appl Environ Microbiol. 2003;69:1435–41.
9. Kolattukudy PE. Bio-Polymer Membranes of Plants–Cutin and Suberin. Science. 1980;208:990–1000.
10. Jetter R, Schlaffer S. Chemical composition of the Prunus laurocerasus leaf surface. Dynamic changes of the epicuticular wax film during leaf development. Plant Physiol. 2001;126:1725–37.
11. Forster-Fromme K, Jendrossek D. Identification and characterization of the acylcyl terpenic enzyme cluster of Pseudomonas citrinovirogenes. FEMS Microbiol Lett. 2006;264:220–5.
12. Viňa A, Murín E. Essential oil composition from twelve varieties of basil (Ocimum spp.) grown in Colombia. J Braz Chem Soc. 2003;14:744–9.
13. O'Brien R. Enzymatic analysis of the pathways of glucose catabolism and gluconeogenesis in Pseudomonas citrinovirogenes. Arch Microbiol. 1975;103:71–6.
14. Hector ML, Fall RR. Multiple acyl-coenzyme A carboxylases in Pseudomonas multiresinovorans. Biochimie. 1976;58:1345–72.
15. Omasits U, Quebatte M, Stekhoven DJ, Fortes C, Roschitzki B, Robinson MD, Hector ML, Fall RR. Multiple acyl-coenzyme A carboxylases in Pseudomonas multiresinovorans. Biochimie. 1976;58:1345–72.
16. Degand N, Carbonnelle E, Dauphin B, Beretti J-L, Le Bourgeois M, Sermet-Gaudelus I, Segonds C, Berche P, Nassif X, Ferroni A. Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Identification of Nonfermenting Gram-Negative Bacilli Isolated from Cystic Fibrosis Patients. J Clin Microbiol. 2006;44:3561–7.
17. Dieckmann R, Graaber I, Kaesler J, Szewzyk U, Von Dohlen H. Rapid screening and dereplication of bacterial isolates from marine sponges of the Sula Ridge by Intact-Cell-MAFFT-TOF mass spectrometry (ICM-MS). Appl Microbiol Biotechnol. 2005;67:539–48.
18. Vorholt JA. Microbial life in the phyllosphere. Nat Rev Microbiol. 2012;10:828–35.
19. Yang CH, Crowley DE, Borneman J, Keen NT. Microbial phyllosphere populations are more complex than previously realized. Proc Natl Acad Sci U S A. 2001;98:3889–94.
20. Tukey Jr H, Tukey Sr H. The loss of organic and inorganic materials by leaching from leaves and other above-ground plant parts. In: Radioisotopes in plant-soil nutrition studies. 1962. p. 289.
21. Morgan J, Tukey Jr H. Characterization of leachate from plant foliage. Plant Physiol. 1964;42:345–53.
56. Freimoser FM, Pelludat C, Remus-Emsermann MNP. Tritagonist as a new term for uncharacterised microorganisms in environmental systems. ISME J. 2015;10(1):1–3.

57. Patrick J, Steains K. Auxin-promoted transport of metabolites in stems of Phaseolus vulgaris L.: auxin dose-response curves and effects of inhibitors of polar auxin transport. J Exp Bot. 1987;38:203–10.

58. Bhatia M, Girdhar A, Tiwari A, Nayanasari A. Implications of a novel Pseudomonas species on low density polyethylene biodegradation: an in vitro to in silico approach. SpringerPlus. 2014;3:497.

59. Miyazaki R, Bertelli C, Benaglio P, Canton J, De Coi N, Gharib WH, Gjoksi B, Goesmann A, Greub G, Harshman K, et al. Comparative genome analysis of Pseudomonas knackmussi B13, the first bacterium known to degrade chloroaromatic compounds. Environ Microbiol. 2015;17:91–104.

60. Sandhu A, Halverson LJ, Beattie GA. Bacterial degradation of airborne phenol in the phyllosphere. Environ Microbiol. 2007;9:383–92.

61. Scheublin TR, Leveau JH. Isolation of Arthrobacter species from the phyllosphere and demonstration of their epiphytic fitness. Microbiologopen. 2013;2:205–13.

62. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A. 1990;87:4576–9.

63. Garrity GM, Bell JA, Lilburn T. Phylum XIV. Proteobacteria phyl. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. Bergey’s Manual of Systematic Bacteriology. 2nd Ed. Volume 2, Part B. New York: Springer; 2005. p. 1.

64. Garrity GM, Bell JA, Lilburn T. Class III. Gammaproteobacteria class. nov. In: Garrity GM, Brenner DJ, Krieg NR, Staley JT, editors. Bergey’s Manual of Systematic Bacteriology, 2nd Ed. Volume 2, Part B. New York: Springer; 2005. p. 1.

65. Orla-Jensen S. The Main Lines of the Natural Bacterial System. J Bacteriol. 1921;6:263–73.

66. Skerman VBD, McGowan V, Sneath PHA. Approved lists of bacterial names. Int J Syst Evol Microbiol. 1980;30:225–420.

67. Winslow CEA, Broadhurst J, Buchanan RE, Krumwiede C, Rogers LA, Smith GH. The Families and Genera of the Bacteria: Preliminary Report of the Committee of the Society of American Bacteriologists on Characterization and Classification of Bacterial Types. J Bacteriol. 1917;2:505–66.

68. Migula W. Über ein neues System der Bakterien. In: Arbeiten aus dem Bakteriologischen Institut der Technischen Hochschule zu Karlsruhe. 1894. p. 235–8.

69. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al. Gene Ontology: tool for the unification of biology. Nat Genet. 2000;25:25–9.

70. Stothard P, Wishart DS. Circular genome visualization and exploration using CGView. Bioinformatics. 2005;21:537–9.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at www.biomedcentral.com/submit