

# **Isolation and characterization of *Pseudomonas chlororaphis* strain ST9; rhizomicrobiota and *in planta* studies**

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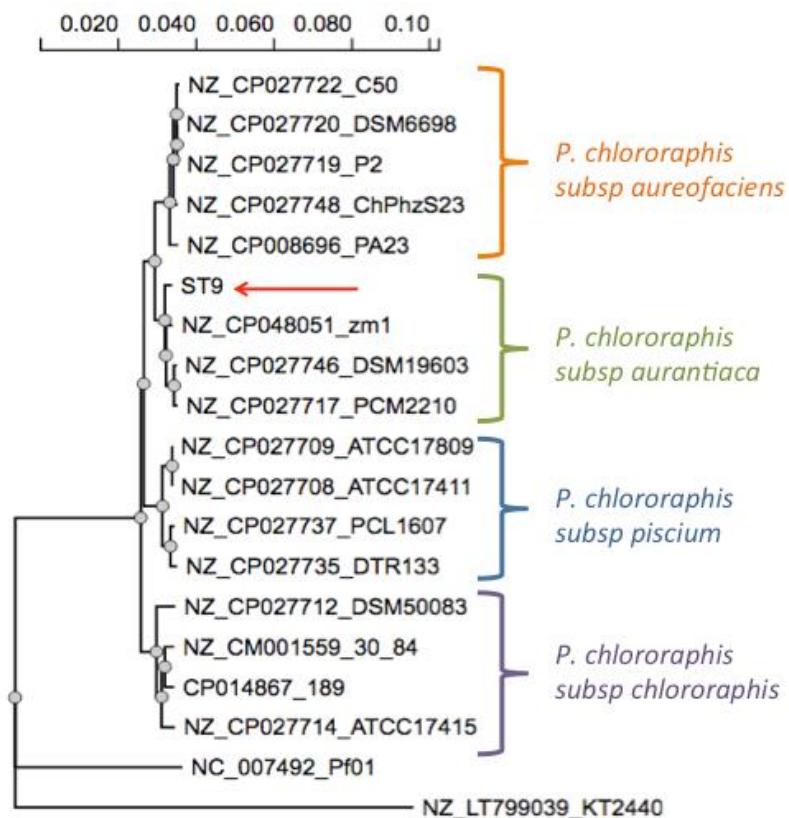
§ Equal Contribution

**Keywords:** rice, plant growth promoting bacteria, qPCR, microbiome, inoculum persistence,  
genome analysis

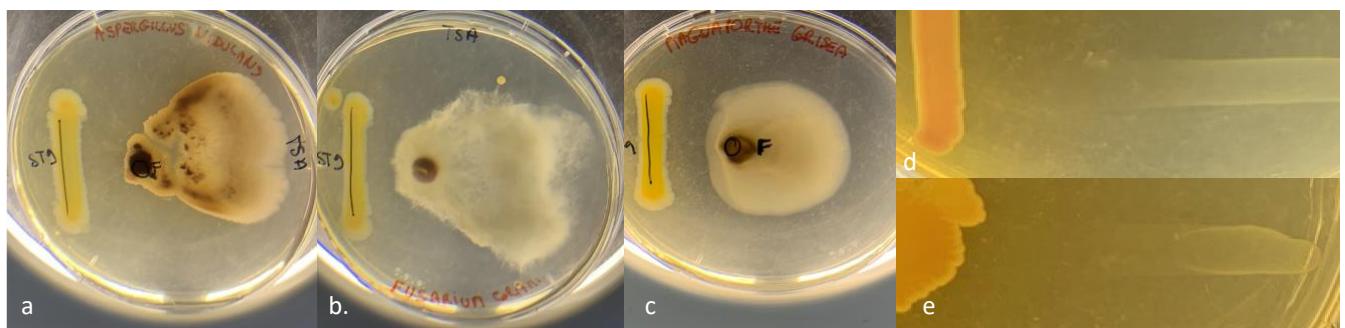
## **This PDF file includes:**

- Supplementary Figures S1-S5
- Supplementary Tables S1 and S2
- Supplementary Material S1
- Reference

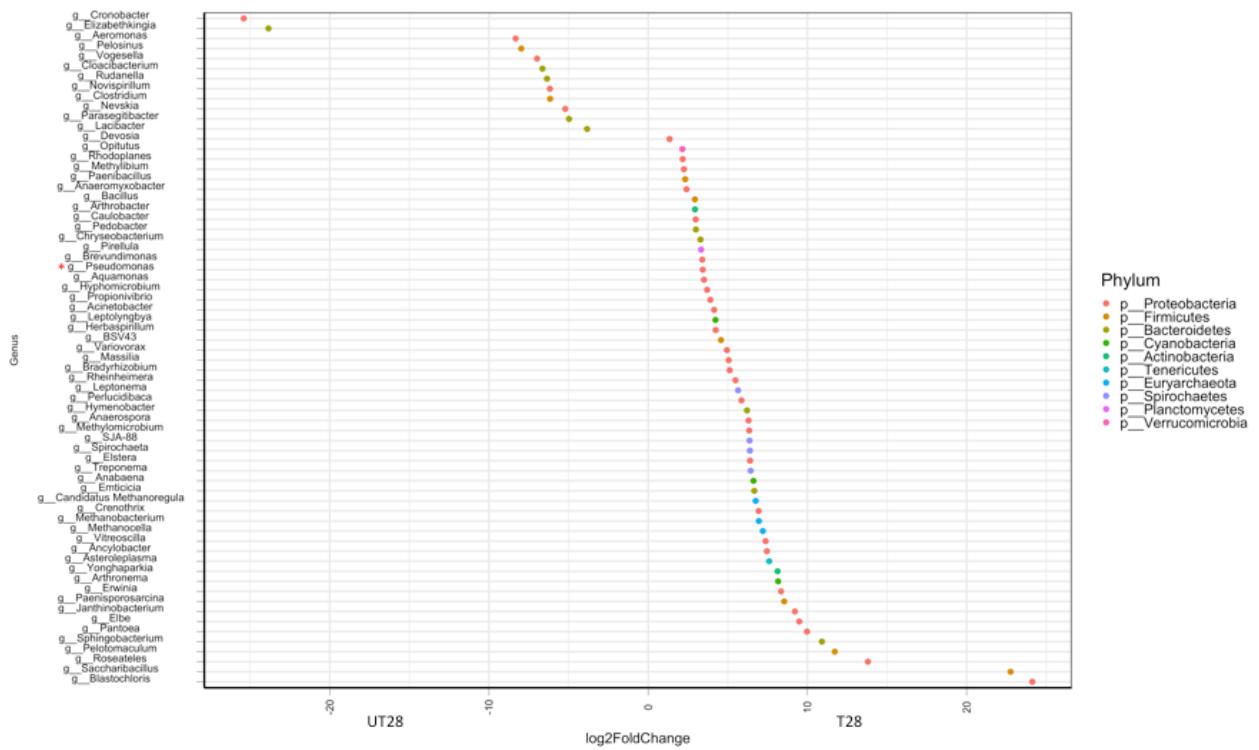
## Supplementary Figures



**Supplementary Figure S1.** Phylogenetic tree reconstructed by the MSLA method based on six concatenated gene sequences (16S rRNA, *recA*, *gyrB*, *rpoD*, *carA*, *atpD* – 9371 nt-) of 17 *Pseudomonas chlororaphis* strains. The strains *P. putida* KT2440 and *P. fluorescens* Pf01 served as outgroups. The 4 subspecies of *P. chlororaphis* cluster separately and ST9 is part of the *aurantiaca* subspecies. The phylogenetic analysis was performed using the NGPhylogeny.fr public platform.

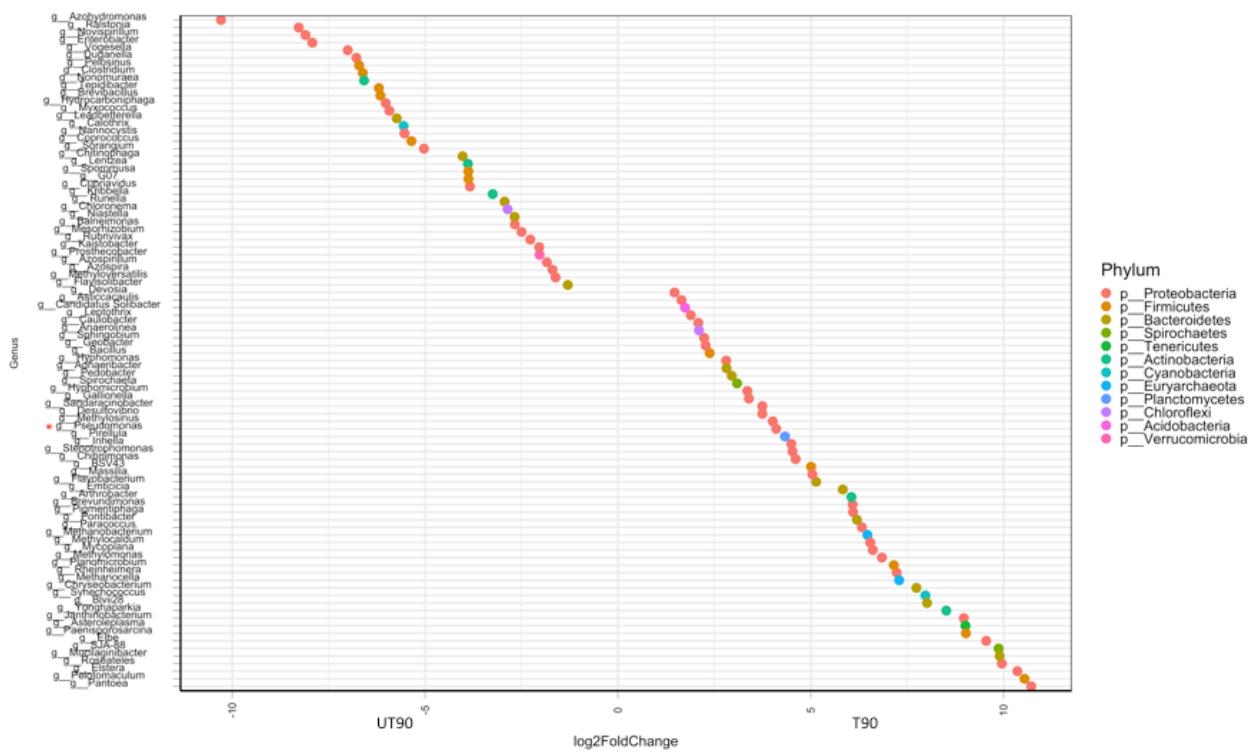


**Supplementary Figure S2.** *In vitro* antimicrobial activity of *P. chlororaphis* ST9. The antifungal activity was tested against a. *Aspergillus nidulans*, b. *Fusarium graminearum* and c. *Magnaporthe oryzae*. The antibacterial activity was verified against d. *Dickeya zeae* and e. *Pseudomonas fuscovaginae*.

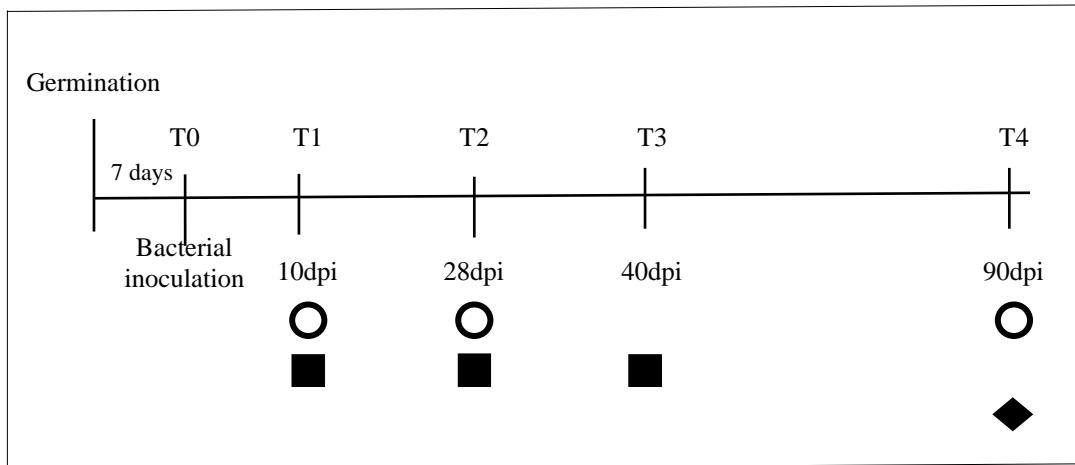


**Supplementary Figure S3.** Differential representation of OTUs between ST9 inoculated and control samples at 28 days post inoculation.

Differential abundance of OTUs between the two groups of tested samples was assessed by fitting a local regression model with a negative binomial distribution to the data and testing for differential abundance with a likelihood ratio test as implemented in the R package DESeq2 [1] in conjunction with the phyloseq package. Taxa are represented as dots in the graph of fold change. A negative log2Foldchange indicates taxa more abundant in untreated samples, while a positive log2Foldchange indicates taxa more abundant in treated samples. Samples with a *p*-value less than 0.0001 and mean representation over all samples higher than 1 are shown. UT28: untreated samples at 28 dpi; T28: ST9 treated samples at 28 dpi.



**Supplementary Figure S4.** Differential representation of OTUs between ST9 inoculated and control samples at 90 days post inoculation. Differential abundance of OTUs between the two groups of tested samples was assessed by fitting a local regression model with a negative binomial distribution to the data and testing for differential abundance with a likelihood ratio test as implemented in the R package DESeq2 [1] in conjunction with the phyloseq package. Taxa are represented as dots in the graph of fold change. A negative log2Foldchange indicates taxa more abundant in untreated samples, while a positive log2Foldchange indicates taxa more abundant in treated samples. Samples with a *p*-value less than 0.0001 and mean representation over all samples higher than 1 are shown. UT90: untreated samples at 90 dpi; T90: ST9 treated samples at 90 dpi.



**Supplementary Figure S5.** Time line of the sampling and use of the samples: ○ bacterial counting and microbiome analysis, ■ plant gene expression analysis, ◆ plant physiological and morphological evaluations.

## Supplementary Tables

Table S1: List of the primers used in this study

Primer name	Primer sequence (5'-3')	Gene name	Putative function	Reference
fD1	AGAGTTGATCCTGGCTCAG	<i>16SrRNA</i>	16S rRNA amplification	[2]
rP2	ACGGCTACCTTGTACGACTT			
518F	CCAGCAGCCCGGTAATACG	<i>16SrRNA</i>	16S rRNA sequencing	[2]
800R	TACCAGGGTATCTAATCC			
OsERS1f	GAAAGGTAGGCTCTCTGAAATC	<i>OsERS1</i>	Ethylene response sensor 1	[3]
OsERS1r	ATGCCGTCGATCAATTACAGTAG			
OsERS2f	CCTCGGGTTCGCTACCAAT	<i>OsERS2</i>	Ethylene response sensor 2	[3]
OsERS2r	GCATGGCGATGGCATCAT			
OsETR2f	CTTAGCAGCACTGGGAGATGA	<i>OsETR2</i>	Ethylene responsive 2	[3]
OsETR2r	TGAGAACCATGAGGCTCTTCA			
OsETR3f	CGAGCTGGCGAATT	<i>OsETR3</i>	Ethylene responsive 3	[3]
OsETR3r	TTAGACAAACAGACCTCCAGCAA			
OsIAA1f	ACCAAGAGCCGCTCAATGAG	<i>OsIAA1</i>	auxin-responsive protein IAA1-like	[4]
OsIAA1r	ATCACACGTGGCGAACATC			
OsIAA4f	GCTCTGCTGGATGGGTATGA	<i>OsIAA4</i>	auxin-responsive protein IAA4	[4]
OsIAA4r	AGGTGATGGCGTCTGAAC			
OsIAA11f	AGTTGTCCATGGCGTTCCA	<i>OsIAA11</i>	auxin-responsive protein IAA11	[4]
OsIAA11r	TGCTCTCCTTCAGCTGCTGAT			
OsIAA13f	CAAGGATGGTACTGGATGCT	<i>OsIAA13</i>	auxin-responsive protein IAA13-like	[4]
OsIAA13r	GATCCTCAAGCGTTGCATGA			
OsIAA14f	CCGTCGCCTATGAGGACAA	<i>OsIAA14</i>	auxin-responsive protein IAA14-like	[4]
OsIAA14r	TTATCCGCAGCTTCTGCAA			

OsACT1f	GTATCCATGAGACTACATACAAC	<i>OsACT1</i>	Actin 1	[5]
OsACT1r	TACTCAGCCTTGGCAATCCACA			
ARF2-like f	GGCCTGAATCAAGTTGGAGATC	<i>OsARF2</i>	Similar to auxin response factor 2	[6]
ARF2-like r	CTATCTGGCCCGCGGAATAGTT			
ERF2-like f	CCGGCAAGGGTAGAGATGGT	<i>OsERF2</i>	Similar to ethylene response factor 2	[6]
ERF2-like r	TCAACATCGAAATCCCAAGAACT			
ERF3-like f	TGCAGCAGCCTATGCAGATC	<i>OsERF3</i>	Similar to ethylene response binding factor 3	[6]
ERF3-like r	CGCGAGGACACTGCTTGAT			
OsISAP1 f	GCAATCCTCATCACACAGCAA	<i>OsISAP1</i>	Multiple stress-responsive zinc-finger protein	[6]
OsISAP1 r	CCCTCTTGGTCTCAGGCTCTCT			
Metallothionein f	CAAAC TGCTCCTGC GGAAAG	<i>Osmetallo-thionein</i>	metallothionein-like protein type 1	[6]
Metallothionein r	ACGACGGTGGCCTTGGT			

Table S2: Fold change at each time point

Gene	Time		
	T1	T2	T3
<i>OsERF2</i>	0.22	0.02*	0.02*
<i>OsMetallothionein</i>	0.11*	0.01*	0.01*
<i>OsIAA1</i>	0.22	0.002*	0.01*
<i>OsIAA11</i>	0.21	0.05	0.43
<i>OsIAA13</i>	2.54	2.81*	0.3
<i>OsIAA14</i>	1.8	10.54*	4.4*
<i>OsISAPI</i>	1.16	3.02*	0.62
<i>OsETR3</i>	1.72	41.8*	1.88
<i>OsARF2</i>	0,69	19,35*	1,16
ERS1	0,3	8,54*	2,04
<i>OsERS2</i>	0,87	22,09*	2,46
<i>OsERF3</i>	0,81	5,68*	0,56*
<i>OsIAA4</i>	0,37	3,22	6,3
<i>OsETR2</i>	0,73	19,27*	2,19

\*: statistically significant different (P< 0.05)

## Supplementary Materials (SM)

### Supplementary Material 1

Sequences used for the taxonomic analysis of *P. chlororaphys* ST9.

NCBI Reference Sequence: NZ\_CP014867.1

Strain: 189

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_CP014867.1\_189

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NCBI Reference Sequence: NZ\_ CP027714.1

Strain: ATCC17415

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_CP027714\_ATCC17415

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NCBI Reference Sequence: NZ\_CP027735.1

Strain: DTR133

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_CP027735\_DTR133

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NCBI Reference Sequence: NZ\_CP027748.1

Strain: ChPhzS23

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_CP027748\_ChPhzS23

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NCBI Reference Sequence: NZ\_CP027720.1

Strain: DSM6698

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_CP027720\_DSM6698

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NCBI Reference Sequence: NZ\_CP027737.1

Strain: PCL1607

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_CP027737\_PCL1607

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RAST server Genome ID: 286.2086

Strain: ST9

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

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NCBI Reference Sequence: NZ\_CP027708.1

Strain: ATCC17411

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_CP027708\_ATCC17411

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NCBI Reference Sequence: NZ\_CP027722.1

Strain: C50

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_CP027722\_C50

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NCBI Reference Sequence: NZ\_CM001559.1

Strain: 30-84

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_CM001559\_30\_84

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NCBI Reference Sequence: NZ\_CP027712.1

Strain: DSM50083

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_CP027712\_DSM50083

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NCBI Reference Sequence: NZ\_CP027717.1

Strain: PCM2210

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_CP027717\_PCM2210

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NCBI Reference Sequence: NZ\_CP027746.1

Strain: DSM19603

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NCBI Reference Sequence: NZ\_CP027709.1

Strain: ATCC17809

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NCBI Reference Sequence: NZ\_CP027719.1

Strain: P2

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_CP027719\_P2

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NCBI Reference Sequence: NZ\_CP008696.1

Strain: PA23

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_CP008696\_PA23

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NCBI Reference Sequence: NC\_007492.2

Strain: Pf01

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NC\_007492\_Pf01

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NCBI Reference Sequence: NZ\_CP048051.1

Strain: zm-1

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_CP048051\_zm1

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NCBI Reference Sequence: NZ\_LT799039.1

Strain: KT2440

Chained genes: 16S rRNA, recA, gyrB, rpoD, carA, atpD

>NZ\_LT799039\_KT2440

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TTCAGCGGCATCCTCAAAGGTGACTACGACCAACCTGCCAGAACAAAGCGTTACATGG  
TCGGCAGCAGTACGAGCGATCGAGAAAGCCAAGAAACTGTAA

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