Defense responses against *Rhizoctonia solani*, interactions with bacterial inoculants and root exudation of antifungal compounds in lettuce are differentially expressed on different soils

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Introduction

- **Biocontrol** of plant pathogens is discussed a promising approach, due to limited availability of conventional control measures (e.g. resistant crop cultivars and efficient pesticides with low eco-toxicological risks).
- However, exploitation of microbial inoculants as biocontrol agents in crop production systems is frequently hampered by inconsistent results at the field scale
- Using the model pathosystem *Lactuca sativa/ Rhizoctonia solani*, this study focused on soil type effects on expression of bottom rot disease severity and biocontrol activity of two bacterial inoculants (Pseudomonas jessenii RU47; Serratia plymuthica 3Re-4-18), investigating plant perfor-mance, plant nutritional status and root exudation of antifungal compounds.

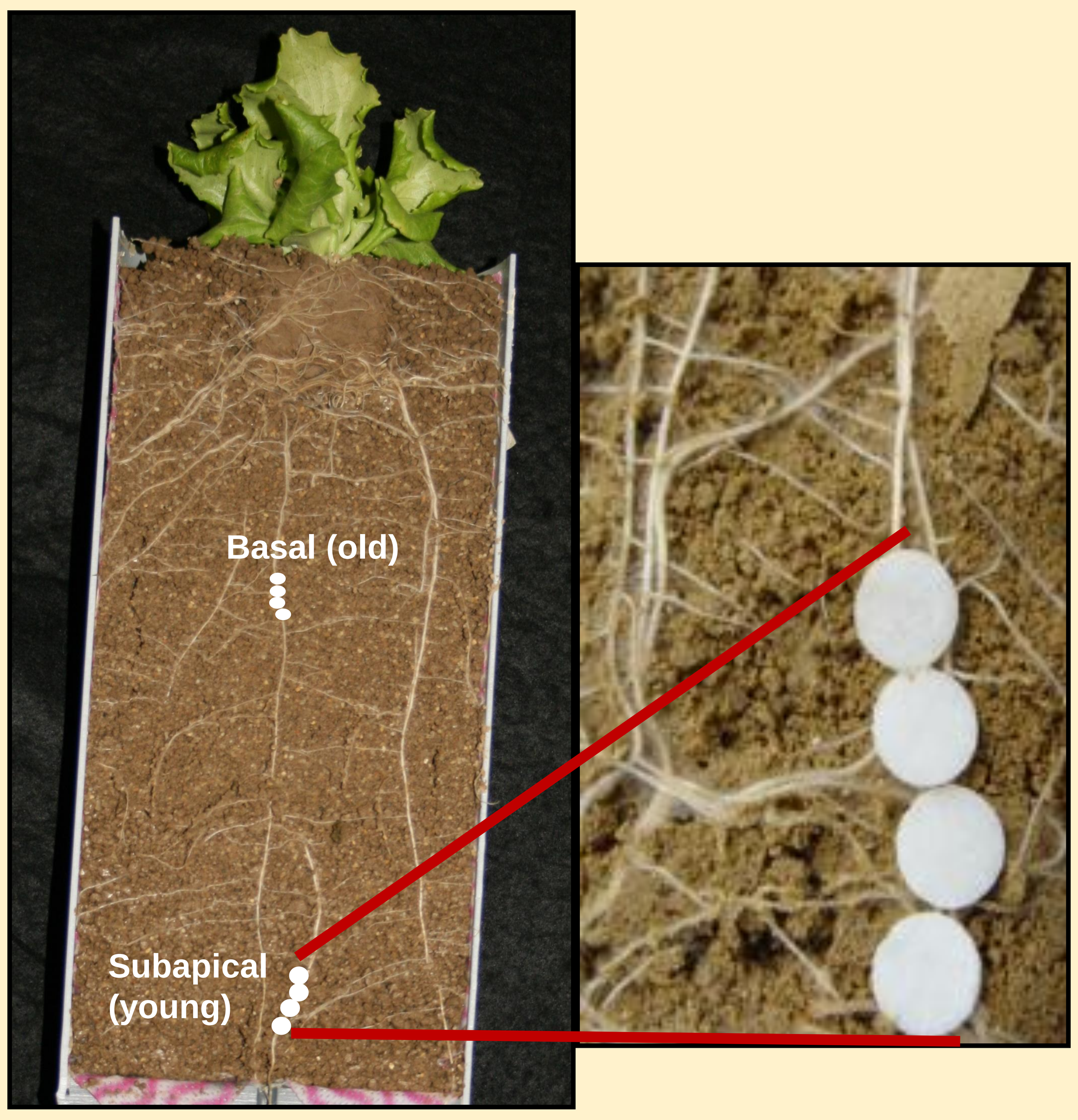

Figure 1. Mini-rhizotrons with *Lactuca sativa* and root exudate collection with sorption filters on subapical (young) and basal (old) root parts.

Experimental setup

- **Model plant:** *Lactuca sativa* L. cv. Tizian on 3 soil types (diluvial sand (DS) pH 6.7, alluvial loam (AL) pH 6.7 and loess loam (LL) pH 7.1).
- **Cultivation of *Lactuca sativa*** in mini-rhizotrons with transparent root observation window until BBCH19) in a growth chamber: 16h light period, 60% rel. humidity and a day/night temperature of 25/28° C, standard NPK fertilisation.
- **Inoculants:** *Pseudomonas jessenii* RU47 and *Serratia plymuthica* 3Re-4-18: seed treatment (250µL/100 seeds) and 20 mLsoil drenching (10^8 cfu mL^{-1}). *Rhizoctonia solani* AG1-IB isolate 7/3: soil inoculation with infected agar blocks (Schreiter et al., 2014).
- **Plant analysis:** Root exudates were collected with sorption filters placed onto the root surface in apical and sub-apical root zones followed by re-extraction and GC-MS analysis (Neumann et al., 2014, Windisch et al., 2017). Plant mineral analysis by attomic absorption spectrometry (AAS)

Neumann G. et al. (2014): Front. Microbiol. 5: 1–6
Schreiter S. et al. (2014): PLoS ONE 9: 1–11
Windisch S. et al. (2017): Agronomy 7, 44: 1-17

Table 1. Inoculation with *Rhizoctonia solani* affected shoot biomass production (g DM $plant^{-1}$) of *Lactuca sativa* grown on diluvial sand and alluvial loam but not on loess loam

Treatment	Diluvial Sand	Alluvial Loam	Loess Loam
Control	0.22 ± 0.2	1.13 ± 0.1 a	1.33 ± 0.5 a
+ *Rhizoctonia*	++	0.70 ± 0.3 b	1.45 ± 0.2 a

++ Plants died within the first week after *R. solani* inoculation

Disease Suppression by Bacterial Inoculants

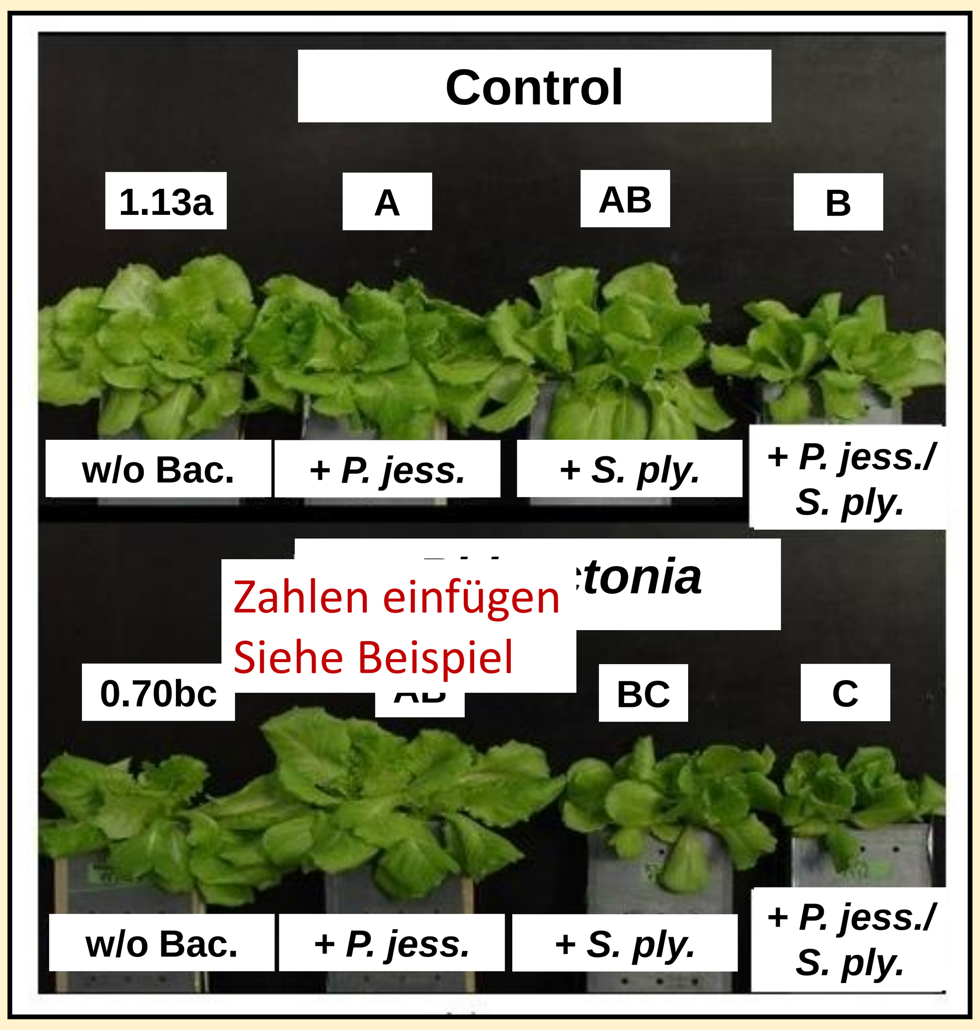

Fig. 2. Shoot Biomass (g pot^{-1}) of *Lactuca sativa*, grown on alluvial loam. Protective effects of bacterial inoculants against R. solani but reduced biomass production by double inoculation.

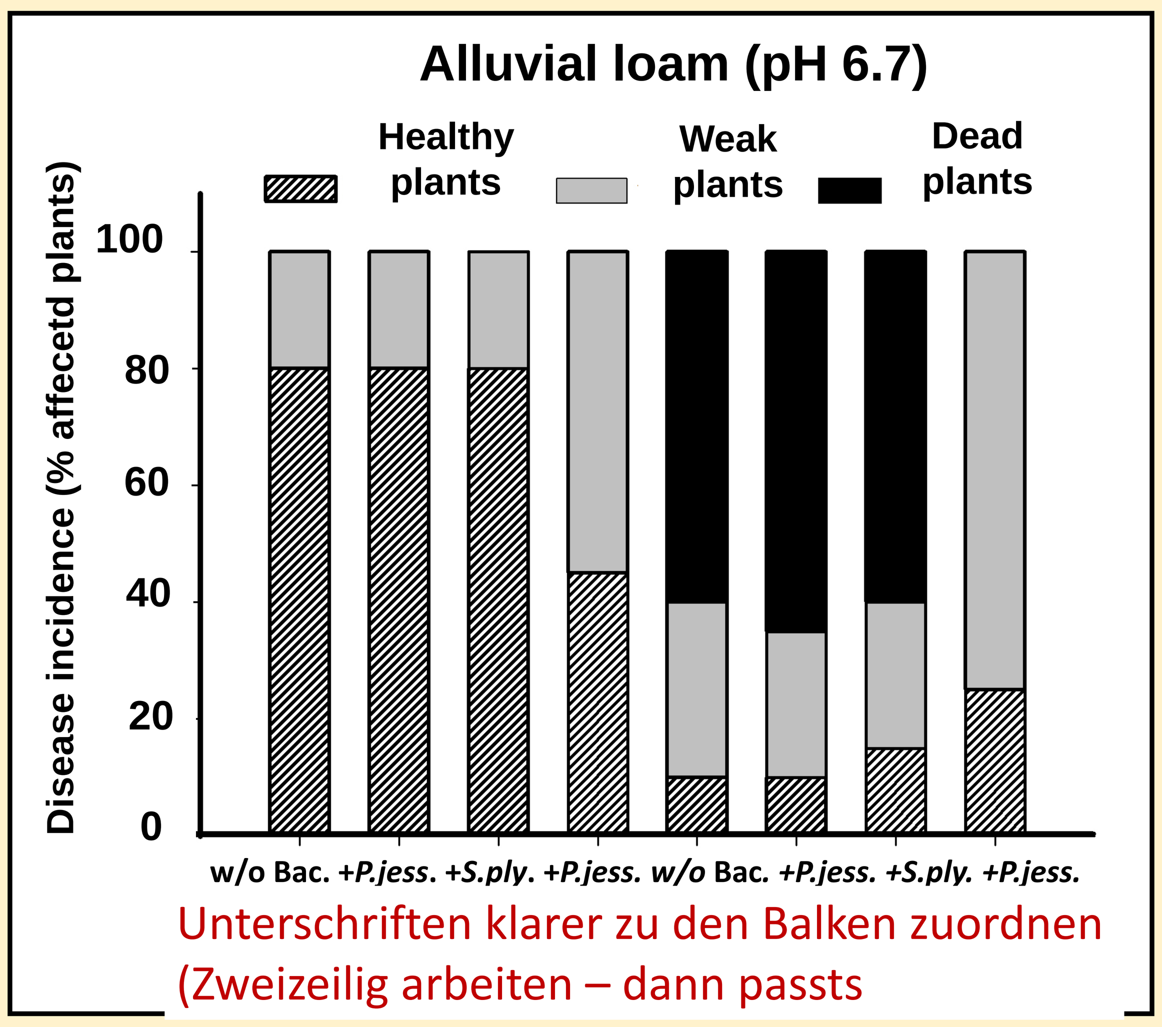

Fig. 3. Visual scoring of disease incidence in *Lactuca sativa* grown on alluvial loam revealed the highest protective effect against *R. solani* by double-inoculation with *Pseudomonas jessenii* and *Serratia plymuthica* (all plants survived)

Tab.2 Benzoic acid in the rhizosphere soil solution affected by microbial inoculants (relative values based on peak areas (GC-MS) minus background levels in bulk soil)

Treatment	Benzoic acid			
	Subapical		Basal	
	Alluvial Loam	Loess Loam	Alluvial loam	Loess Loam
Control	3.92 ^{a}	5.00 ^{a}	2.77 ^{a}	6.30 ^{a}
+ *R. solani*	13.07 ^{b}	5.53 ^{a}	10.97 ^{b}	8.12 ^{a}
+ *P. jessenii*	12.84 ^{b}	10.54 ^{b}	12.98 ^{b}	13.58 ^{ab}
+ *S. plymuthica*	13.08 ^{b}	10.49 ^{b}	12.75 ^{b}	9.58 ^{ab}
+ *P. jessenii* / *S. plymuthica*	12.84 ^{b}	N.d.	12.63 ^{b}	N.d.
+ *R. solani* + *P. jessenii*	10.75 ^{b}	9.62 ^{b}	13.65 ^{b}	15.53 ^{b}
+ *R. solani* + *S. plymuthica*	11.71 ^{b}	8.63 ^{b}	12.68 ^{b}	15.79 ^{b}
+ *R. solani* + *P. jessenii* / *S. plymuthica*	12.14 ^{b}	N.d.	11.31 ^{b}	N.d.

N.d = not determined. Different characters indicate significant differences.

- ***R. solani* induces root exudation of antifungal compounds (benzoic acid) only on the soil with high pathogen conductivity (alluvial loam) but not on the non-conductive loess loam (Tab.1). Pre-inoculation with bacterial antagonists induces benzoic acid exudation on both soils.**

Side Effects of Bacterial Inoculants

Tab 3. Micronutrient concentrations in shoots (mg kg^{-1} DM) of *Lactuca sativa* grown on alluvial loam

Treatment	Zn	Mn	Fe
Control	35.00 ^{ab}	30.12 ^{b}	73.41 ^{a}
+ *R. solani*	41.52 ^{a}	50.23 ^{a}	65.34 ^{ab}
+ *P. jessenii*	42.42 ^{a}	40.23 ^{a}	72.34 ^{a}
+ *S. plymuthica*	43.71 ^{a}	39.94 ^{a}	74.65 ^{a}
+ *P. jessenii* / *S. plymuthica*	31.56 ^{b}	27.23 ^{bc}	54.67 ^{b}
+ *R. solani* + *P. jessenii*	39.73 ^{a}	37.51 ^{ab}	70.23 ^{a}
+ *R. solani* + *S. plymuthica*	35.61 ^{ab}	29.97 ^{b}	63.34 ^{ab}
+ *R. solani* + *P. jessenii* / *S. plymuthica*	33.45 ^{b}	23.45 ^{c}	53.23 ^{b}

In presence of *R. solani*, double inoculation with *P. jessenii* and *S. plymuthica* significantly decrased the micronutrient status of *Lactuca sativa* close to the deficiency range.

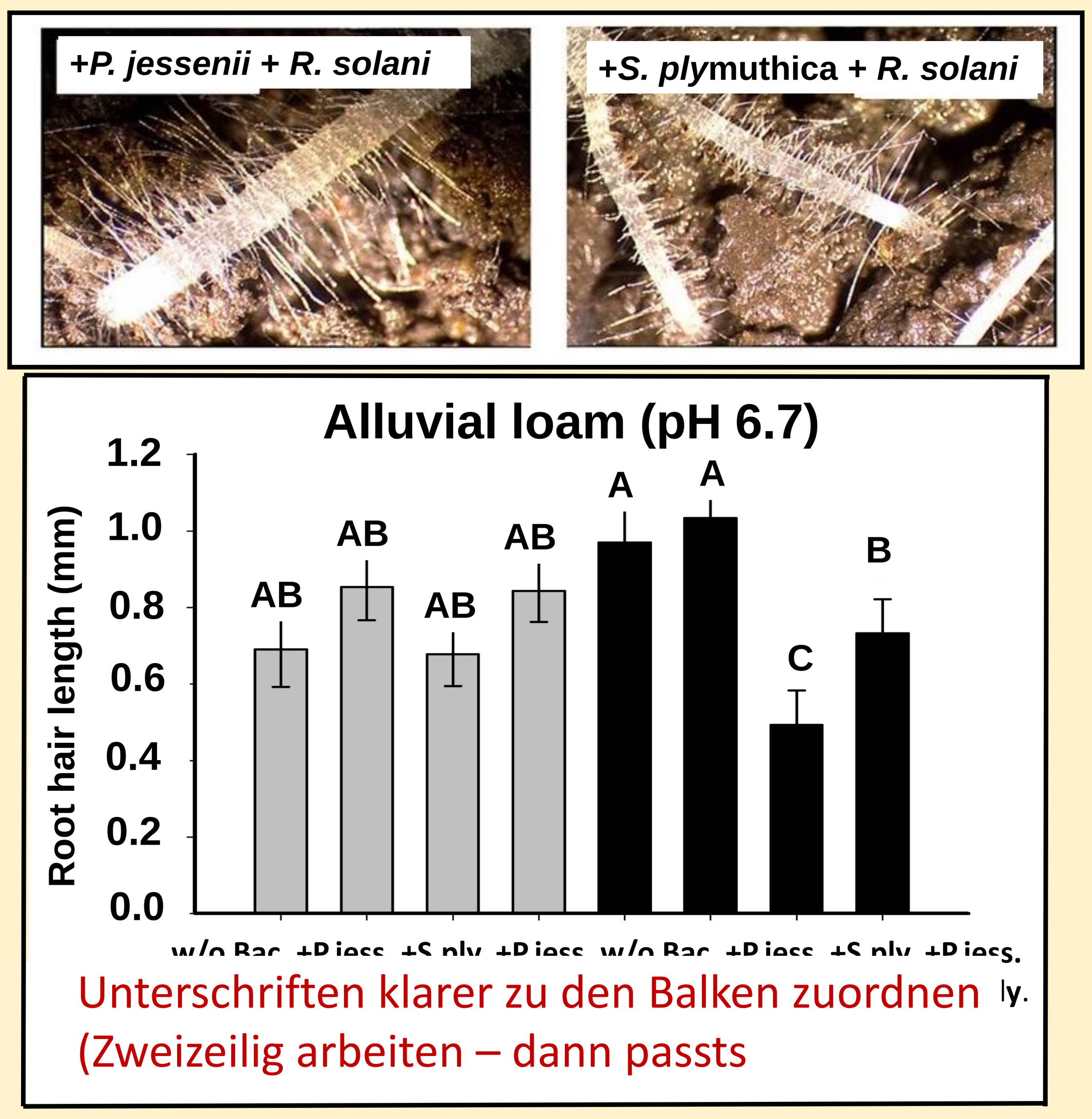

Fig 4. Root hair development of *Lactuca sativa* important for micro-nutrient acquisition was inhibited by *Serratia plymuthica*

Conclusions

- Disease severity and the expression of biocontrol effects are infuenced by soil properties.
- Disease severity declined in the order DS>AL>LL soil.
- Pathogen inoculation and pre-inoculation with bacterial inoculants significantly increased the release of antifungal root exudates in soil specific manner.
- However also negative side effects of bacterial inoculants (reduction of shoot growth, root hair development and a limited micronutrient status of the host plant must be taken into consideration after double-inoculation.

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