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Phylogenetic placement of RrF4 to closest related Agrobacterium biovar I, II, and III strains. Phylogenetic tree (a) and pairwise average nucleotide identity (ANI) values (b) of strains based on core genes of the selected strains as determined by means of the comparative genomics tool EDGAR (Blom et al., 2009). Reference strains are assigned to the Agrobacterium biovar I: strain C58 (genomovar G8; Goodner et al., 2001), strain WRT31 (genomovar G1; Mondy et al., 2013), strain H13-3 (genomovar G1; Wibberg et al., 2011), strain Arch5 (Henkel et al., 2014), biovar II: R. radiobacter K58 and R. etli CIAT 652, and biovar III: R. vitis S4. The analysis was performed in the comparative genomic tool of EDGAR (Blom et al., 2009). Genomes of reference strains were obtained from GeneBank of NCBI Respective genome Acc. numbers are given in brackets: C58 (AE007869.2 - AE007872.2), WRT31 (CM002024, CM002025), H13-3 (CP002248 -CP002250), LBA4213 (Ach5) (CP007225- CP007228), K58 (CP000628- CP000632), S4 (CP000633 - CP000639), and CIAT 652 (CP000133, CP001075- CP001077) (including Acc. number of plasmid sequences).



Increase in Arabidopsis root and shoot fresh weight (FW) upon inoculation with RrF4. Roots of seven-day-old seedlings were dip-inoculated with RrF4 ($OD_{600} = 1$) or mock-treated (con) with 10 mM MgSO₄ 7H2O. Seedlings were grown under short-day condition. (a) Shoot fresh weight at 21 dpi in soil-sand mix; (b) shoot fresh weight at 21 dpi in vermiculite-sand mixture. (c) Shoot and (d) root fresh weight at 14 dpi on half strength MS medium. (e) Lateral root formation on half strength MS medium induced by RrF4 as compared to mock treatment (f). Error bars indicate standard error, based on three independent biological replicates. Asterisks indicate statistical significance (Student's *t*-test p<0.01).



Colonization pattern of GUS-expressing RrF4 at 5, 7, and 14 dpi in wheat (**a-c**) and Arabidopsis (**d-f**) roots visualized after X-Gluc treatment. Roots of three-day-old wheat or seven-day-old Arabidopsis seedlings were inoculated with GUS-tagged RrF4 cells (OD₆₀₀ = 1.4, wheat, OD₆₀₀ = 1.0, Arabidopsis) and grown on half strength MS medium. Blue color indicates GUS-tagged bacterial cells. Scale bar = 1mm.



Comparison of the colonization pattern of *Rr*F4 and its fungal host *P. indica* in the meristematic-, elongation-, and maturation zone of Arabidopsis roots. While colonization by *P. indica is* mostly restricted to maturation zone II, *Rr*F4 additionally colonized the elongation zone, maturation zone I, and meristematic zone. Image was modified after the figure 1 published by Jacobs *et al.* (2011).



Comparative gene expression analysis in barley roots upon inoculation with RrF4 and P. *indica*. Roots of three-day-old barley seedlings were dip-inoculated with RrF4 and P. *indica*, respectively. Expression of genes was determined by qPCR and relative to barley ubiquitin. Relative expression values for control plants, RrF4 and P. *indica* treated plants were calibrated to the three dpi time point of the untreated control (set to 1). Bars and error bars show mean values and standard error of three independent biological experiments. Asterisks indicate the statistical significance obtained by One way ANOWA performed with the Dunnett's test by comparing treatment groups against controls (p<0.05%).



Systemic expression of plant hormone-responsive genes in Arabidopsis wild type. Three-weekold Arabidopsis roots were pre-treated for 3 days with *Rr*F4 and then leaves were challenged with *Pst*. Expression of *PR1*, *ERF1*, *VSP2*, and *PDF1.2* genes in leaves were determined by qPCR relative to Arabidopsis ubiquitin gene expression. Relative expression values for control plants and *Rr*F4 treated plants were calibrated to the control 0 hpi time point (set to 1). Mean values and standard error of three independent biological experiments are given. Asterisks indicate statistical difference using the Student's t-test p< 0.05.



*Rr*F4-induced systemic resistance against bacterial streak disease in wheat. Roots of three-dayold seedlings were dip-inoculated with *Rr*F4 ($OD_{600} = 1.4$) and grown in soil. At 21 dpi, leaves were spray-inoculated (challenge inoculation) with *Xanthomonas translucens* pv. *translucens* (*Xtt*). (a) Disease severity determined on a scale based on diseased leaf areas at 5 and 7 days after challenge inoculation (dpci). (b) Disease symptoms at 7 dpci. All values are related to the non-pretreated (*Rr*F4-free) control (7 dpci) which was set to 100. *Rr*F4-pretreated plants show reduced *Xtt* symptoms compared with non-pretreated plants. (c) Disease severity as assessed by chlorophyll content at 7 dpci. Error bars indicate standard error based on three independent biological replicates using 15 plants for each treatment. Asterisks indicate statistical difference between the control and pretreated plants (Student's *t*-test **p<0.01; ***p<0.001). Different letters at top of the bars indicate significant differences determined by One Way ANOWA based on the tukey test (p<0.05).