The potential of bacteria for the control of common bunt in organic wheat production

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Background

In organic wheat production, *Tilletia caries* is one of the most important seed-borne pathogenic fungi due to its high spreading potential and the toxicity of its spores. It causes the disease common bunt or stinking smut (see Fig. 1). Since the introduction of chemical seed dressings, common bunt of wheat is globally well controlled, but it is still a major threat in organic seed production where chemical or synthetic seed dressings are prohibited. The aim of this work was to evaluate the potential of newly isolated bacteria to protect wheat against common bunt.

Figure 1 Typical symptoms of the disease common bunt. Left are the bunt balls developed in the wheat ears instead of kernels, in the middle a wheat plant grown under controlled conditions showing chlorotic spots on their leaves caused by *T. caries*, on the right black spore powder inside the bunt balls.

Experiments

Eight bacterial strains (six *Pseudomonas*, one *Streptomyces* and one *Bacillus*) were chosen and tested for

- potential inhibition of *T. caries* spore germination on medium.
- effects on wheat germination (stimulation or phytotoxic effects).
- reduction of disease symptoms in *Tilletia*-infected wheat.

Effects of bacterial strains on *Tilletia* spore germination

In the control treatment, 59% of the spores germinated. The strain *Pseudomonas chlororaphis* R47 completely inhibited *T. caries* spore germination (Fig. 2A). Another *P. chlororaphis* strain, which is commercialized as Cerall®, a seed dressing against *T. caries*, reduced germination only to 47%. The *Streptomyces* strain S01 had a slight but significant effect. While the other bacterial strains showed no significant inhibition of spore germination. However, most strains modified the hyphal structures, leading to the formation of branched or helix-like structures (Fig. 2B).

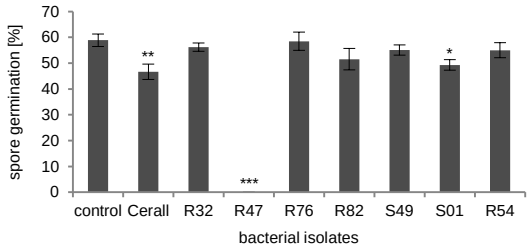

Figure 2A Effect of eight bacterial strains on the spore germination of *T. caries*. Bacteria suspensions in 0.9% NaCl were spotted on agar drops on microscope slides. Thereafter, a *Tilletia* spore solution was spotted onto the agar drops and germination was determined after 7 days. NaCl solution was used as negative control. Significant differences from the control are depicted by stars (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Student's t test, n = 8-9, $n_{ctrl} = 15$. Standard error bars are shown.

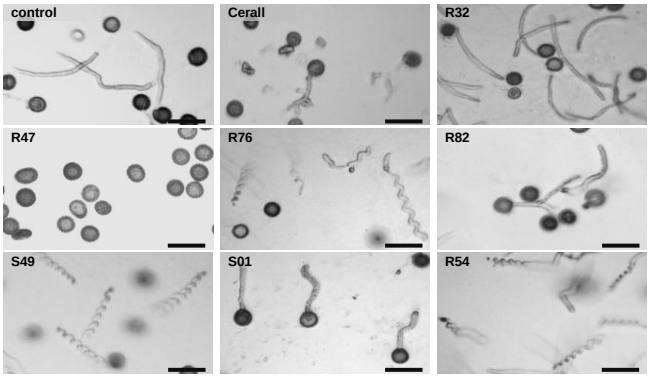

Figure 2B Representative pictures of bacterial effects on the hyphal structure and germination of *T. caries* spores. Pictures were taken after one week of incubation. Magnification 160x, bars = 90 µm.

Effects of bacterial strains on wheat germination

Seeds germinated normally when inoculated with the bacterial strains. Moreover, we observed a protective effect of the six *Pseudomonas* strains against various black head mold development during wheat seed germination (Fig. 3). The *Bacillus* (R54) and the *Streptomyces* (S01) strains showed no effects.

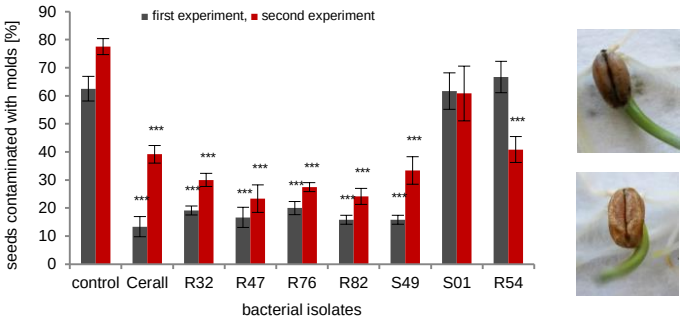

Figure 3 Bacterial effects on different black head molds on Apogee wheat seedlings in germination boxes (each containing 30 seeds). Grey: first experiment, red: second experiment. Significant differences from the control are depicted by stars (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Student's t test, n = 4. Standard error bars are shown. Right, Representative picture of germinated wheat seeds. Top: control seedling (treated with NaCl), black molds are visible; bottom: seedling treated with bacterial strain R47.

Effects of bacterial strains on symptom development

We selected three *Pseudomonas* (Cerall®, R32, R47) and one *Bacillus* strain (R54) for a pot experiment with *Tilletia*-infected wheat. None of the strains could inhibit disease progression (Tab. 1), which was detected by chlorotic spots on the leaves (Fig. 4).

seed treatment	infected plants
NaCl solution	0%
NaCl solution + *T. caries*	97%
Cerall® + *T. caries*	90%
R32 + *T. caries*	100%
R47 + *T. caries*	100%
R54 + *T. caries*	100%

Table 1 Apogee seeds treated with different bacterial solutions (Cerall®, R32, R47, R54) or 0.9% NaCl control solution and 1 mg of *T. caries* spores per seed except in the first mode. High infection rates of plants with common bunt symptoms were visible developing during one to three weeks after vernalization time of 7 days.

Figure 4 Wheat leaves of three weeks old wheat plants. On the left side two leaves of an infected plant with *T. caries* showing the typical chlorotic spots of the early symptoms. On the right side two leaves of an uninfected control plant.

Summary

T. caries spore germination was completely inhibited by *P. chlororaphis* R47, while the commercialized seed dressing against *T. caries*, Cerall®, did not reach this inhibitory dimensions. No phytotoxic effects were observed when wheat seeds were coated with the strains. Moreover, the *Pseudomonas* strains significantly reduced black mold development. However, in the pot experiment, no protective effects against *T. caries* were observed.

Outlook

The amount of *T. caries* spores used in the pot experiment was very high (1 mg/seed). Further experiments will be done with lower spore density to investigate potential effects of the bacteria. Now that the early symptom method is established, we will also be able to test a higher number of strains for protective effects against *T. caries* and to improve the formulation of the strains.

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