# Screening concept for plant-microbe interactions on the Styrian oil pumpkin as basis for an advanced breeding strategy

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## Introduction


**Figure 1:** The Styrian oil pumpkin: (A) plants, (B) ripe fruits and (C) seeds lacking lignification of the seed coat.

The dark-green, healthy pumpkin seed oil of the **Styrian oil pumpkin** (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb.) is of traditional use in Austria and has also become popular in the international gourmet cuisines (Figure 1A and 1B).

Due to the lack of lignification of the seed coat (Figure 1C) this crop is highly susceptible to various pathogens during germination. **Biotic and abiotic stress factors** influence the performance of the adult plants as well.

Collectively known as the **plant microbiome**, plant-associated microbes can help plants to fend off diseases, stimulate growth and promote stress resistance. Additionally they influence crop yield and quality (Berg et al. 2013). As a high cultivar-specificity has already been shown for interactions with pathogens it is likely, that there also exists a high cultivar-specificity on the beneficial plant-microbe interactions.

**The development of a strategy to screen for beneficial plant-microbe interactions should support breeding of new cultivars that are better capable to exploit the beneficial indigenous microbial community as well as additionally applied biocontrol agents.**

## Materials & Methods

**Screening methods** already established for the evaluation of biocontrol agents should be used for the assessment of differences in **plant-microbe interactions** of oil pumpkin cultivars. For this purpose five microbial model strains, listed in Table 1, should be applicated to the seeds of four homozygous **oil pumpkin breeding lines** as well as on a **F1 hybrid** with different characteristics.

**Table 1:** Model strains for the purpose as beneficial microbes.

| Strain | Reported results |
|---|---|
| *Serratia plymuthica* S13 | Increased seedling emergence of field grown pumpkins by up to 109 % (Fürnkranz et al. 2012) |
| *Serratia plymuthica* 3Rp8 | Significant growth promotion in greenhouse trials (Adam, 2015, unpublished data) |
| *Lysobacter gummosus* L101 and *Paenibacillus polymyxa* PB71 | Significant suppression of powdery mildew, reproducible increases in harvest yields (Fürnkranz et al. 2012) |
| *Trichoderma velutinum* G1/8 | Suppression of *Didymella bryoniae in vitro* (Adam, 2015, unpublished data) |

The **evaluation of the plant-microbe interactions** include:

- a **visualization** of root colonization patterns involving determination of cell counts: for confocal laser scanning microscopic analysis, model strains (*Serratia plymuthica* S13 and 3Rp8 as well as *Trichoderma velutinum* G1/8) were transformed with rhizosphere-stable vectors hosting different fluorescent proteins
- **greenhouse experiments** to evaluate seed germination and early plant development after introduction of varying microbial communities
- **field trials** for the evaluation of pathogen suppression on adult plants and the influence of a shift in the microbial community on the yield
- **metagenome analyses**, **microbial fingerprints** and **fluorescence in situ hybridization microscopy** of field samples to determine the enrichment of different taxonomic taxa depending on the cultivar.

## Results

Seed priming with ***Serratia plymuthica* biocontrol strains** under axenic conditions resulted in a high **abundance** of the bacteria ($10^9$ cfu $g^{-1}$ fresh weight) on the roots and on the leaves.

Figure 2A shows a densely colonized root, visualized by confocal laser scanning microscopy. Nevertheless an uneven **distribution** of the bacteria within the root system was detected (brighter areas of the roots in Figure 2C).

The **migration** of bacteria and fungi in the soil and along the roots was tested in compartment petri dishes (Figure 2D) showing a fast migration of bacteria on the root surface and a slow migration from the roots to the leaves of the plants.

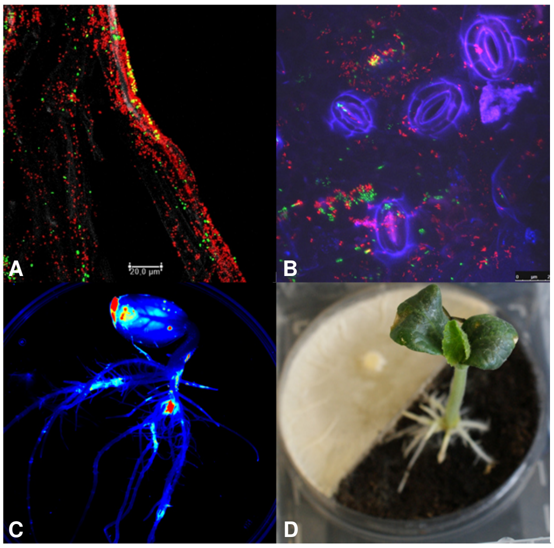


**Figure 2:** Screening methods of plant-microbe interactions: (A) root and (B) young leaf surface colonization visualized by using fluorescent strains and confocal laser scanning microscopy, (C) visualization of the colonization of the entire root system under the Bio-Rad ChemiDoc XRS-System and (D) test of the transport of bacteria on the plant by using two compartment petri dishes.

## Discussion & Perspectives

- As plants normally have to deal with a complex microbial environment, the experiments should be repeated in heavily infested soils in combination with the fluorescent biocontrol strains.
- Further, those and other methods should be used to characterize the microbe interactions with the four inbreed lines and the F1 hybrid. In this concern **possible differences in the interactions depending on the cultivar** should be detected and evaluated.
- Fingerprint and metagenome analysis of field samples of the different breeding lines as well as of plants with and without a biocontrol treatment grown on fields should further lead to a better understanding of the plant-microbe interactions of different cultivars.

**References:**

Berg G, Zachow C, Müller H, Philipps J, Tilcher R. 2013. Next-generation bio-products sowing the seeds of success for sustainable agriculture. Agronomy 2013. 3: 648-656.

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