Current Biology

Nutrient- and Dose-Dependent Microbiome-Mediated Protection against a Plant Pathogen

Highlights

- Leaf-associated microbiota confer protection against pathogen *Pseudomonas syringae*
- Degree of protection depends on the initial dose of microbiota being applied
- A 12-member community applied at low doses was sufficient to reduce pathogen growth
- Fertilizer application to host plants abolished the microbiome-mediated protection

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In Brief

Berg and Koskella find that inoculation of tomato plants with leaf-associated microbiota can confer protection against *P. syringae* under controlled experimental conditions but that the degree of protection varies across microbiome communities and depends on both the microbiota inoculum dose applied and the plant's inorganic nutrient status.



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Nutrient- and Dose-Dependent Microbiome-Mediated Protection against a Plant Pathogen

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SUMMARY

Plant-associated microbial communities can promote plant nutrient uptake, growth, and resistance to pathogens [1-3]. Host resistance to infection can increase directly through commensal-pathogen interactions or indirectly through modulation of host defenses [4-6], the mechanisms of which are best described for rhizosphere-associated bacteria. For example, Arabidopsis plants infected with the foliar pathogen, Pseudomonas syringae pathovar tomato (Pst), increase their root secretion of malate, which attracts Bacillus subtillis to the roots and leads to a stronger host response against Pst [7]. Although there are numerous examples of individual defensive symbionts (e.g., [8]), it is less clear whether this type of protection is an emergent property of whole microbial communities. In particular, relatively little is known about whether and how the presence of phyllosphere (above-ground) microbial communities can increase host resistance against pathogens. In this study, we examined the ability of augmented tomato phyllosphere microbiomes to confer resistance against the causal agent of bacterial speck, Pst. Across five independent experiments, the augmented phyllosphere microbiome was found to decrease pathogen colonization. Furthermore, the dose of commensal bacteria applied affected the degree of protection conferred, and although the effect is dependent on microbial composition, it is not clearly related to overall bacterial diversity. Finally, our results suggest that resources available to the phyllosphere microbial community may play an important role in protection, as the addition of fertilizer abolished the observed microbiome-mediated protection. Together, these results have clear relevance to microbiome-mediated protection within agricultural settings and the use of plant probiotics to increase disease resistance.

RESULTS AND DISCUSSION

The microbiomes of both plants and animals have been shown to provide beneficial host functions [1, 3, 9, 10], including conferring

resistance against pathogens either directly through microbemicrobe interactions [4, 5, 11] or indirectly through alteration of host defenses [2, 6, 12, 13]. Microbe-mediated disease resistance in plants has primarily focused on the rhizosphere and the surrounding soil microbial community [5, 14], where soils that suppress pathogen growth (i.e., disease-suppressive soils) do so by increasing microbe-pathogen competition for resources in the rhizosphere [15]. Although rhizosphere bacteria have been shown to increase resistance to infection [2, 14], little has been done to study how the phyllosphere community can impact host resistance, even though the phyllosphere is likely to be important for pathogens infecting aerial portions of plants, such as Pseudomonas syringae pathovar tomato (Pst). We therefore set out to examine the ability of augmented tomato phyllosphere microbiomes to confer resistance against Pst, the causal agent of bacterial speck.

Phyllosphere Microbiome Provides Protection against Pathogen Colonization

To examine how phyllosphere (above ground) microbiota of tomato plants can affect Pst colonization of leaves, we first infected plants sprayed with different doses of "leaf wash" containing phyllosphere microbes from field-grown tomato leaves or with sterile buffer. Plants were sprav inoculated with either undiluted or 85% diluted concentrations (referred to herein as "dose") of each of six independently generated leaf wash inocula one week before infection (n = 3 plants per treatment). We then pressure inoculated three leaves per plant with Pst and measured bacterial densities after 24 hr in both infected and uninfected leaves using droplet digital PCR (see STAR Methods). In our uninfected leaves, spray-inoculated plants had higher densities of bacteria compared to plants sprayed with sterile buffer (Figure 1A; Mann-Whitney test comparing 16S copy number; Z = -2.138; p = 0.032), confirming that our inoculation methods were successful in establishing a phyllosphere community.

For leaves subsequently inoculated with *Pst*, we observed an effect of phyllosphere augmentation on *Pst* density, where spray-inoculated plants hosted lower densities of *Pst* than those inoculated with buffer (Figure 1B; Z = -2.084; p = 0.035). More fine-grained analysis uncovers a significant interaction effect between leaf wash source and dose on *Pst* density in leaves (general linear model [GLM] with source and dose as fixed effects; $F_{5,23} =$ 9.037; p < 0.001) but no main effect of either source ($F_{5,23} = 1.068$; p = 0.404) or dose ($F_{1,23} = 0.750$; p = 0.395). This interaction effect was not observed for overall bacterial densities (16S copy number) of uninfected leaves ($F_{5,23} = 1.585$; p = 0.204), where we Download English Version:

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