

Highlight

Pollen has a microbiome: implications for plant reproduction, insect pollination and human allergies

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Flower pollen has a microbiome (Colldahl and Nilsson, 1973; Junker and Keller, 2015; Obersteiner *et al.*, 2016). As Manirajan and colleagues (2016) report in the previous issue of *EM*, pollen from four different species of flowering plant (birch, rape, rye and autumn crocus) each harbor on their surfaces a complex bacterial microbiome. Over 1000 different bacterial species comprising 100 families could be identified by DNA sequencing of nucleic acid extracted from the pollen samples of these four plants. Forty-four bacterial species, representing 18 families, from the pollen samples were culturable (and hence were alive) including *Enterobacteriaceae* which are common inhabitants of the gastrointestinal tract of humans and other animals. Curiously, although the plants from which the pollen was studied co-existed in the same geographical region, the microbiomes of each plant species exhibited a distinct pattern of bacterial diversity. Examination of the pollen grains by scanning electron microscopy revealed the presence of individual bacterial cells attached to the pollen wall or as microcolonies within a patch of a tiny biofilm. The numbers of bacteria adherent to flower pollen was rather impressive, ranging from about 10^6 (birch) to about 10^9 (autumn crocus) cultivatable bacteria per gram of pollen.

The discovery that grains of pollen carry a diverse population of live bacteria has implications for our understanding of the design of the reproductive tissues of flowering plants, the relationship between a flowering plant and its insect pollinator, and the mechanism of human pollen allergy.

Plants, like animals, must co-exist with microbes. Both live with a population of 'friendly' micro-organisms (commensals) that occupy various niches both within their

internal milieu and on their body surfaces. And both must defend themselves from pathogens that can disrupt their health, fertility and survival. Both plants and animals utilize antimicrobial peptides to help them manage the microbes they encounter within their environment (Zasloff, 2002). Antimicrobial peptides are generally short cationic amphipathic molecules (12–80 amino acids), ribosomally encoded and expressed in all tissues into which a microbe might gain entrance. In the case of bacteria, AMPs generally act by binding electrostatically to the exposed bacterial membrane, disrupting its integrity, leading to rapid death of the target. Defensins – AMPs which are constrained into a specific tertiary structure by pairs of cystine bridges – represent the class most commonly found in plants (Tam *et al.*, 2015).

One of the most surprising observations in recent years in plant immunology is the extraordinary investment made by the plant in the expression of amphipathic peptides on the pollen coat and the female reproductive tract that exhibit the tertiary structure and characteristic amino acid composition of defensin-like AMPs. About 800 genes encoding AMP-like cysteine rich peptides have been identified in *Arabidopsis* of which about 300 closely resemble defensins in structure (Silverstein *et al.*, 2005). Of these 300, more than 80 exhibit close homology to a defensin-like peptide (PCP-A1) previously isolated from the pollen coat of *Brassica* (Doughty *et al.*, 1998), and at least 200 of the putative 800 cysteine rich peptides are expressed within the embryo sac (Jones-Rhoades *et al.*, 2007). The AMP-like peptides of the pollen coat appear to play a role in preventing a flower's self-fertilization, by interacting with receptors on the tissues of the pistil (Nasrallah, 2002). The AMP-like peptides secreted by the synergid cells of the embryo sac direct the pollen tube from the surface of the pistil to the ovary, where another group of AMP-like peptides induces rupture of the pollen tube and release of the male gametes (Higashiyama and Takeuchi, 2015). It's not unreasonable to hypothesize that the diversity of microbes on the pollen surface is likely constrained, in part, by the AMPs expressed on the pollen coat. The use of ovarian derived AMPs as pollen tube chemo-

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attractants insures that microbes that remain adherent to the pollen tube will face an ever increasing AMP concentration as it extends through the tissues of the pistil and approaches the female gamete. It seems remarkable that the AMPs of the pollen grain and those secreted by the ovary must also be working together, in that microbes permitted to adhere on the pollen grain should be microbial species easily decimated by the defenses of the ovary. How would fertilization fare in the presence of environmental microbes that managed to escape this putative antimicrobial barrier?

With the discovery of a pollen's microbiome comprising viable bacteria it also becomes quite reasonable to imagine that insects involved in pollination must also be capable of an immune defense against the microbes of the pollen they collect. Insects are 'hardwired' with respect to their antimicrobial defenses, in the sense that the AMPs they express are genome encoded, as are the receptors that recognize the microbes encountered. As a consequence insect species are constrained to inhabit niches that are populated by a microbiome with which they have coevolved. Perhaps insect-flower pollination relationships are in part dictated by the immune capacity of the insect and the specific microbiome investing the surface of the pollen grain. Would the application of an agricultural pesticide/herbicide/fungicide that altered the normal plant microbiome subject the insect pollinators of that plant species to microbes against which they had no effective immune defense? Might this explain, in part, why bees are dying worldwide?

That pollen is associated physically with bacteria likely explains why pollen allergy is so common (Colldahl and Nilsson 1973; Obersteiner *et al.*, 2016). We now understand that when pollen lands on our respiratory mucosa our immune system is presented with both the antigen (pollen), and an adjuvant (the bacterial cells). Antigen-presenting cells that are positioned in our body at a barrier in contact with microbes must experience the antigen in the presence of a microbial constituent (or 'PAMP') to fulfill their function properly. Only in that context will the mucosal dendritic cell or macrophage (the usual cells) effectively carry out its role of presenting the foreign antigen to circulating lymphocytes. In a sense, pollen is a perfect mucosal vaccine. Would passively or actively immunizing an individual against pollen-associated microbes prevent pollen specific allergy? Do those suffering from allergy to the pollen of a specific flower carry circulating antibodies directed at the microbiome of that species of pollen?

Perhaps it was 'obvious' that pollen has its own microbiome. However, the implications of this discovery are far reaching, and have profound impact on our understanding of the immune response to pollen by both plants and animals, including humans.

References

- Colldahl, H., and Nilsson, L. (1973) Possible relationship between some allergens (pollens, mites) and certain microorganisms (bacteria and fungi). A morphological study using the scanning electron-microscope. *Acta Allergol* **28**: 283–295.
- Doughty, J., Dixon, S., Hiscock, S.J. Willis, A.C., Parkin, I.A., Dickinson, H.G., (1998) PCP-A1, a defensin-like Brassica pollen coat protein that binds the S locus glycoprotein, is the product of gametophytic gene expression. *Plant Cell* **10**: 1333–1347.
- Higashiyama, T., and Takeuchi, H. (2015) The mechanism and key molecules involved in pollen tube guidance. *Annu Rev Plant Biol* **66**: 393–413. doi:10.1146/annurev-arplant-043014-115635
- Jones-Rhoades, M.W., Borevitz, J.O., and Preuss, D. (2007) Genome-wide expression profiling of the Arabidopsis female gametophyte identifies families of small, secreted proteins. *PLoS Genet* **3**: 1848–1861. doi: 10.1371/journal.pgen.0030171
- Junker, R.R., and Keller, A. (2015) Microhabitat heterogeneity across leaves and flower organs promotes bacterial diversity. *FEMS Microbiol Ecol* **91**: fiv097. doi:10.1093/femsec/fiv097
- Manirajan, B.A., Ratering, S., Rusch, V., Schwiertz, A., Geissler-Plaum, R., Cardinale, M. (2016) Bacterial microbiota associated with flower pollen is influenced by pollination type, and shows a high degree of diversity and species-specificity. *Environ Microbiol* **18**: 5161–5174. DOI: 10.1111/1462-2920.13524
- Nasrallah, J.B. (2002) Recognition and rejection of self in plant reproduction. *Science* **296**: 305–308. doi:10.1126/science.296.5566.305
- Obersteiner, A., Gilles, S., Frank, U., Beck, I., Haring, F., Ernst, D. (2016) Pollen-associated microbiome correlates with pollution parameters and the allergenicity of pollen. *PLoS One* **11**: e0149545. doi:10.1371/journal.pone.0149545
- Silverstein, K.A., Graham, M.A., Paape, T.D., and VandenBosch, K.A. (2005) Genome organization of more than 300 defensin-like genes in Arabidopsis. *Plant Physiol* **138**: 600–610. doi:10.1104/pp.105.060079
- Tam, J.P., Wang, S., Wong, K.H., and Tan, W.L. (2015) Antimicrobial peptides from plants. *Pharmaceuticals (Basel)* **8**: 711–757. doi:10.3390/ph8040711
- Zasloff, M. (2002) Antimicrobial peptides of multicellular organisms. *Nature* **415**: 389–395. doi:10.1038/415389a