Title: Plant microbiota affects seed transmission of phytopathogenic micro-organisms

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Introduction

Seeds, defined in this opinion paper as sexually derived structures of spermatophytes, are involved in the vertical transmission of microorganisms from one plant generation to another and act consequently as a primary source of inoculum for the crop. A variety of micro-organisms such as plant-growth promoting agents, plant or animal pathogens have been isolated from the seed surfaces or the seed tissues of various plant species. These seed-associated micro-organisms could represents transient colonizers of the seed habitat or alternatively be transmitted to the plantlet and in consequence influence seedling-associated microbial assemblages. Therefore, one should differentiate between seed-borne and seed-transmitted micro-organisms.

Seed transmission of micro-organisms can have various detrimental effects on seed physiological quality including seed discoloration or decrease of germination rate. Moreover, the sanitary quality of seed could also be impacted by transmission of pathogen through contamination of seeds with mycotoxins or Shiga toxins. From an epidemiological point of view, seed transmission of phytopathogenic microorganisms represents an important mean of pathogen dispersion and is therefore significant in emergence of disease. Even low level of seed contamination is sufficient to lead to efficient colonization of plants by bacterial pathogens (Darrasse et al., 2007). Moreover, seed transmission of plant pathogenic agents can occur on nonhost plant. For instance, the bacterial pathogen of Brassicas, *Xanthomonas campestris* pv. *campestris* (*Xcc*), is efficiently transmitted from mother plant to seeds of bean and from bean seeds to seedlings (Darrasse et al., 2010, Darsonval et al., 2008). This nonhost carriage could serve as a potential reservoir of pathogenic agents in new planting areas and may contribute to an increase of the gene pool available for recombination. For all these reasons, exploring plant-pathogen interactions during the plant reproductive stage is of interest for control of plant disease.

Vertical transmission guarantees persistence of a micro-organism from parents to offspring. To manage vertically transmitted pathogens one should either favor exclusion of pathogens from the mother plant or treat the seed to eliminate them. Chemical treatments applied on seed-producing
crops or as seed treatments are efficient methods to control fungal pathogens. In contrast these chemical-base methods are unsatisfactory for bacterial plant pathogens. Therefore control of seed-transmitted bacterial pathogens relies either on alternative seed treatments such as thermotherapy or on prophylactic measures performed on crops and seed samples. Nevertheless, none of these strategies guarantees pathogen-free seeds. Biological control is a promising option but has been historically hampered by variation in efficacy of the employed microbial strains, which could partly explained by the empirical selection of biocontrol agents. The survey of seed-associated microbial assemblages presented in this opinion paper should provide novel options to select biocontrol agents.

**Processes contributing to efficient seed transmission of phytopathogenic agents**

Seeds of different plant species vary greatly in term of anatomy and morphology (Singh & Mathur, 2004). Despite these variations in structure, mature seeds could be schematically divided into 3 main compartments: (i) the embryo, which is composed of the embryonic axis and the cotyledon(s), (ii) storage tissues such as the perisperm and the endosperm and (iii) the seed coat (also known as testa). It is noteworthy that non-endospermic seeds such as Fabaceae stored nutritive resources within the cotyledons and are therefore devoided of storage tissues.

Numerous micro-organisms including bacteria and fungi have been isolated from the seed coat, while contamination of the endosperm and the embryo seed is less frequent (Maude, 1996; Singh & Mathur, 2004). The location of micro-organisms within seed tissues is dependent of the stage at which the seed is colonized by these micro-organisms. Early colonization of the developing seeds could occurred through via the xylem or nonvascular tissue of the mother plant (Maude, 1996). These internal seed transmission pathways are restricted to few pathogenic micro-organisms and endophytic microbes. Alternatively, developing seeds could also be invaded by microorganisms through the floral pathway via the stigma of the mother plant (Maude, 1996). The internal and floral pathways result in colonization of all seed tissues from the embryo to the testa. In
contrast, late colonization of the mature seeds through contact of the seed with microorganisms present on fruits or threshing residues is usually restricted to the seed coat (Singh & Mathur, 2004). Since this seed transmission pathway is more permissive than the internal or floral pathway, microbial assemblages associated to seed surface are probably more diverses than the microbiota of seed internal tissues.

Understanding the relative importance of seed transmission pathways in relation to specific disease is a prerequisite for developing efficient control methods. Indeed location of microorganisms in seed can affect transmission efficiency to developing seedlings. One the one hand, location of any micro-organism within the embryo guarantees its transfer to the seedling and therefore a successful seed transmission. On the other hand, micro-organisms present on the surface of the embryo or in other tissues of the seed (e.g. endosperm or perisperm, testa) have yet to colonize the seedlings. Indeed, micro-organism has not only to survive to multiple anthropogenic processes such as harvest, cleaning, treatment or storage of seeds but also to survive intense microbial competition near the site of exudation once the seed germinate. Therefore successful colonization of seedling can be dependent on a minimum bacterial population size of 1 x 10^2 CFU per seed (Darrasse et al., 2007). Providing that this minimal inoculum density is reached, it seems that transmission of phytopathogenic microorganisms from seed to seedlings is relatively permissive in both compatible and incompatible interactions. For example, the transmission rates of Clavibacter michiganensis pv. michiganensis, Pseudomonas syringae pv. tomato and Xanthomonas vesicatoria on tomato seedlings is around 50% for each phytopathogenic micro-organisms (Guimbaud et al., unpublished results). This transmission rate can reach on average 80% for X. fuscans subsp. fuscans (Xff) strains on bean seedlings (Guimbaud et al., unpublished results) and 100% for Acidovorax citrulli on watermelon plantlets (Dutta et al., 2012). Moreover, multiplication rates of Xcc and Escherichia coli on bean seedling are not significantly different from multiplication rate of Xff, suggesting that whatever the type of interaction, bacteria behaved similarly at emergence (Darrasse et al., 2010). This similar colonization pattern could be explained by the absence of
induction of plant defenses during emergence (Darrasse et al., 2010). In contrast, during development, seeds respond to pathogen presence through activation of plant defenses and subsequent repression of seed maturation pathways (Terrasson et al., 2015). Based on these results it is tempting to conclude that transmission of micro-organisms from seed to seedling is relatively permissive in comparison to the transfer from mother plant to seeds. Therefore development of control methods aiming to decrease the efficiency of plant to seed transmission of plant pathogen could represent interesting strategies for production of seeds with optimal sanitary quality. Alternatively, selection of biocontrol agents possessing key molecular determinants required for successful persistence during germination and emergence are also of interest for exclusion of phytopathogenic agents on plantlets.

**Potential influences of seed-associated microbial assemblages on seed transmission of plant pathogen**

While, the host immune system is undoubtedly an important environmental filter that prevents the establishment of plant pathogen, host-associated microbial assemblages may also restrain this invasion. The resistance of a microbial community to invasion is usually correlated to its level of diversity as a result of enhanced competition for resources within species-rich community (Mallon et al., 2015). Hence, assessing the microbial diversity associated to seeds and the processes involved in assembly of seed-associated microbial communities are important preliminary steps for designing new biocontrol-based methods.

There is an abundant literature relating to isolation of micro-organisms from seeds of various plant species through classical microbiology techniques. Conversely few studies performed an in-depth investigation of the composition of seed-associated microbial assemblages with culture-independent approaches. Recent profiling of seed-associated microbial assemblages performed on various plant species has revealed that the seed microbiota contains on average less bacterial and fungal taxa than the microbial community associated to the rhizosphere and comparable level of
diversity than the phyllosphere (Barret et al., 2015, Klaedtke et al., 2015, Links et al., 2014). Interestingly most of seed-associated bacterial and fungal OTUs (operational taxonomic units) are linked to species whose members can be easily recovered from culture-dependent approaches. Although some microbial taxa such as *Pantoea* or *Alternaria* are frequently identified in seed of various plant species, an important variation in composition of microbial assemblages exists between seed samples. For example, the number of microbial entities (expressed as OTUs) observed on seeds ranged from 10 to 500 OTUs depending on the molecular marker and analytical workflow employed (Barret et al., 2015, Links et al., 2014). According to the samples analyzed, the composition of seed-associated microbial assemblages is not driven by the plant genotypes (Barret et al., 2015). However, structuration of fungal assemblages could be explained, in part, by the geographic location of the production region. In order to confirm or infirm this observation, the relative influence of the terroir and the host genotypes on the structure of the seed microbiota were assessed on five bean cultivars cropped in two distinct production regions (Klaedtke et al., 2015). The structure of seed-associated fungal assemblages is mostly determined by the production region. This finding may have implications for restricting seed transmission of phytopathogenic fungi through selection of specific production area, where the presence of these plant pathogens have not been detected.

In contrast to results obtained for seed-associated fungal assemblages, variance in bacterial assemblage structure is neither explained by the production region nor the host genotype (Klaedtke et al., 2015). Therefore one might expect that neutral processes such as assembly history may determine the structure of seed-associated bacterial assemblages. It could thus mean that the first bacterial taxa that colonize the seed is probably excluding other equivalent taxa sharing the same functional potential for this habitat. Therefore, inoculation of plant at flowering stage with microorganisms having a high overlap in resource use are likely to restrict subsequent colonization of phytopathogenic micro-organisms and could be used as a promising biological treatment strategy.

As stated earlier, understanding the nature, succession and activities of seed-borne
microorganisms is relevant for determining its successful transmission to the next plant generation. Seed associated micro-organisms must display great physiological adaptation capacity to the changing conditions encountered during seed developmental stages as well as during seed germination. Functions such as adhesion, resistance to hydric and osmotic stresses, quorum sensing and secretion of microbial effectors were demonstrated as key determinants for the transmission to seeds by fungal pathogens such as *Alternaria brassicicola* (Pochon *et al.*, 2013) or bacterial pathogens such as *Xanthomonas* spp. (Darsonval *et al.*, 2009, Darsonval *et al.*, 2008) or *Acidovorax* (Johnson & Walcott, 2013; Tian *et al.*, 2015). In addition, a number of broad functional categories such as chemotaxis, attachment, carbohydrate use and iron acquisition are necessary for successful colonization of germinating seeds by endophytes (Truyens *et al.*, 2015).

The dynamics of different seed-associated microbial assemblages was recently investigated on germinating-seeds and seedlings of numerous plant genotypes (Barret *et al.*, 2015). Based on sequences of different bacterial and fungal markers, we found that fungal and bacterial diversity was markedly decreased during the emergence (**Figure 1**). This reduction of microbial diversity within seedling reflected an increase in relative abundance of some bacterial (e.g. *Pantoea* and *Pseudomonas*) and fungal taxa (e.g. *Cladosporium* and *Alternaria*) possessing fast growing abilities and diverse diet breadths. The enrichment of these microbial taxa on seedling is ultimately associated with the extinction of transient seed colonizers that were initially detected as low population sizes on seeds and germinating seeds (Barret *et al.*, 2015). Therefore for any micro-organism an efficient seed transmission is clearly dependent of a minimum inoculum threshold (Darrasse *et al.*, 2007) along with a great physiological adaptation capacity to the changing conditions encountered during germination and emergence.

**Conclusions – Future directions**

Seed transmission of microorganisms is the primary source of inoculum for the plant and accordingly may play a crucial role on plant growth and plant health. To date, the structure of seed-
associated microbial assemblages and the regulators of assemblage structure have been overlooked. Preliminary studies of microbial assemblages associated to seeds of various plant species have unveiled a relative low microbial diversity in comparison to other plant habitats such as the phyllosphere and the rhizosphere with the gradual enrichment of general seed colonizers during germination and emergence (Figure 1). These seed-associated microbial assemblages seem to be structured by a combination of deterministic and stochastic processes. How are microbial entities assembled throughout the seed development stages (from fertilization to maturation drying; Figure 1)? This is currently unknown and deserves to be tested experimentally through profiling of microbial assemblages on different host plants. In theory, successional changes occurring over the seed development stages within the microbial community should ultimately result in a stable state community. This theoretical framework is particularly relevant for mature seeds that have low moisture content and are almost metabolically inactive, which suggest that associated microorganisms are probably dormant and thus are not interacting anymore with each other.

The stability of a microbial community could be impacted by a number of events, called disturbances that could be of chemical (e.g. fungicide), physical (e.g. temperature changes) or biological (e.g. invader) natures. The response of seed-associated microbial community to biological disturbance caused by seed transmission of phytopathogenic agents is of great interest for proposing biocontrol-based strategies. Futures experiments should assess the influence of distinct microbial invaders differing in their seed transmission pathways (systemic, floral and external) on the composition of seed-associated microbial assemblages. These studies could highlight a number of positive and negative associations between the invaders and the resident member(s) of the seed microbiota, which might be related to mutualistic or competitive interactions between these entities. As most of the members of the seed microbiota are cultivable on synthetic media, isolation of strains representative of these resident members potentially interacting with the pathogenic agent should be relatively straightforward. The invasion success of the plant pathogen will be first tested in laboratory microcosm containing these microbial strains and subsequently assessed during co-
inoculation experiments performed \textit{in planta}.

According to multiple metagenomics studies, it seems that assembly of host-associated microbial community is based on its functional potential rather than on its species composition. Indeed, species having similarities in ecological function such as resource consumption probably compete for nutrient and space and therefore rarely coexist in the same ecological niche. Therefore it seems also important to assess the functional potential of the seed microbiome at various seed and seedlings development stages. Metagenomics- and metatranscriptomics-based approaches are undoubtedly of interest for assessing the genetic content and the gene expression pattern of these microbial assemblages. Abundance comparison of the proteins encoded by seed-associated assemblages to those encoded in the germinating-seed and seedling microbiota would reveal insights into traits involved in the establishment of microbes in the young plants. These traits might be again useful indicators for selection of microbial inoculant possessing biocontrol activities.

References


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**Figure 1: Seed transmission of microbial assemblages at different plant development stages**

Community profiling of microbial assemblages associated to developing seed (**A**), seed (**B**), germinating seed (**C**) and seedling (**D**). Microbial richness (presented here as bacterial and fungal OTUs) and relative abundance of microbial taxa (presented here as bacterial and fungal phyla) has been assessed for stages B, C and D (Barret *et al.*, 2015). However the composition of microbial assemblages associated to developing seeds (**A**) is currently unknown. Notably, the relative influence of the systemic (1) floral (2) and external (3) pathways in seed transmission of the plant microbiota remained to be determined.
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Community profiling of microbial assemblages associated to developing seed (A), seed (B), germinating seed (C) and seedling (D). Microbial richness (presented here as bacterial and fungal OTUs) and relative abundance of microbial taxa (presented here as bacterial and fungal phyla) has been assessed for stages B, C and D (Barret et al., 2015). However the composition of microbial assemblages associated to developing seeds (A) is currently unknown. Notably, the relative influence of the systemic (1) floral (2) and external (3) pathways in seed transmission of the plant microbiota remained to be determined.