Mechanisms of migration of Paraburkholderia terrae BS001 in the mycosphere

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Chapter 2

Mechanisms and ecological implications of the movement of bacteria in soil

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Submitted for publication
Abstract

The extent of translocation of bacteria through soil strongly affects parameters of bacterially-mediated bioremediation and biocontrol. The main strategies that bacteria use for their dispersal are scale-dependent: they are either growth- or cell organelle-driven (small scale, active) or water/wind/vector-organism-driven (large scale, passive). The active modes of dispersal (growth, swimming, swarming and twitching motility) are limited to the scale of (connected) soil pores or aggregates. In contrast, water- or wind-driven transport dominates large-distance bacterial dispersal. Remarkably, the association of bacteria with other (vector) organisms, in particular plant roots, fungal hyphae, and moving organisms like earthworms, assists them in the crossing of soil matrix discontinuities. We posit that an “underground transport web” exists in soil, which is plant-, fungal- and/or soil animal-driven. This web facilitates bacterial dispersal, which has strong implications for the occupancy of novel ecological niches and nutrient cycling dynamics. The effects of this underground transport web are examined.
2.1 Introduction — the importance of bacterial dispersal for differentiation processes and soil functioning

Numerous studies have demonstrated that soil constitutes an enormous reservoir of biological entities that, together, exhibit enormous diversity. This reservoir includes populations of bacteria, fungi, protozoa, nematodes, earthworms, arthropods and plants, among other organisms (Bardgett and van der Putten, 2014; Bender et al., 2016). Across the soil biota, bacteria, in the light of their extremely high numbers, stand out as key inhabitants. Bacteria are the primary players in the soil biogeochemical cycles, such as those of carbon, nitrogen, sulfur and phosphorus (Long et al., 2016). These cycles are crucial for soil health and fertility, as they recycle materials and provide nutrients for plant growth. Bacteria are also involved in bioremediation processes. However, due to their small cell sizes, bacteria act within very small spheres of influence (Kuzyakov and Blagodatskaya, 2015), and they require dispersal mechanism(s) to broaden their zones of influence across the soil microhabitats. Such bacterial dispersal through soil can take place via either active or passive processes, or by a combination of these two.

Active bacterial movement in soil (along soil surfaces) can occur in various ways (Henrichsen, 1972; Jarrell and McBride, 2008). First, bacterial flagella can support two types of motility: (i) swimming motility over a surface with a sufficiently thick fluid film, and (ii) swarming motility at a surface with a limited fluid layer. In addition, type four pili (T4P) mediate the so-called twitching motility, which is highly localized and is driven by the extension and retraction of T4 pilus proteins. Then, bacterial cells can perform another type of movement, called gliding motility, on specific surfaces in the soil matrix. Gliding motility relies on a focal adhesion complex, and works in the absence of both flagella and T4P. Lastly, bacterial cells can be “pushed” forward by expansive force in a growing culture in which cells delay their timing of septum formation and division, with the joined actions of surfactants. This type of motility has been denominated sliding motility.

In addition to the active modes of bacterial cell spread through soil, cells can also be moved passively in soil, e.g. by water flow or even by wind (in dry top soils). In line with this, a somewhat neglected area of study addresses the capacity of specific bacterial types to “hitchhike” along with other
organismal types, being carried by the latter through the soil. Such organisms range from protozoa, nematodes and earthworms to soil fungi and plant root systems. A suite of recent studies has provided support for this emergent area of research (Daane et al., 1996; Kohlmeier et al., 2005; Warmink and van Elsas, 2009; Glaeser et al., 2016).

Motivated by the recent advances in this research area, we here first examine the conditions in soil that critically affect bacterial motility. Then, we address the prevailing types of bacterial motility in soil, and examine to what extent these, depending on the soil ecological context, drive bacterial dispersal. Moreover, we propose the existence of an “underground transport web” that includes fungal hyphal networks, plant roots and earthworms, and describes their interconnections. This transport web is perceived to give a major thrust to bacterial translocation through the soil matrix, with direct implications for soil processes. Finally, we examine the potential ecological effects of bacterial dispersal in soil.

2.2 The soil pore network, water properties and bacterial dispersal

Soil moisture content is an essential factor that determines bacterial cell motility. For instance, *Pseudomonas aeruginosa* Migula was shown to move (20 mm in 24 h) in soil under relatively wet, but not under dry conditions (Griffin and Quail, 1968). The level of connectivity of the water-filled (versus air-filled) pores in the soil has been invoked as an explanatory factor influencing cell motility. For instance, the air-filled pores between soil particles represent structural disconnections that impair cell motility, while ones filled with water bridge the gaps in a continuous manner. The pores in the soil matrix are classified on the basis of the pore size (defined in terms of equivalent cylindrical diameter, ECD, Hayashi et al., 2006), into residual pores (ECD<0.2 μm), fine storage pores (0.2<ECD<6 μm), coarse storage pores (6<ECD<60 μm) and transmission pores (60<ECD<600 μm) (Hayashi et al., 2006). The transmission and coarse storage pores have dimensions that exceed those of bacterial cells and, if not water-filled, effectively impair cell passage. Thus, pore size distribution exerts a critical influence on bacterial motility in soil. In contrast to actual pore median size, pore neck sizes are
important; if these are smaller than bacterial cell sizes, this is likely to restrict the rate of bacterial movement (Andreoglou et al., 2003).

The pore size distribution is intrinsically linked to the soil texture, with a coarser (sandy) soil having a larger proportion of large pores when compared to a finer (clay) soil. Thus, soil texture directly affects bacterial motility in soil, first on the basis of the soil pore network characteristics and secondly due to the forces of attraction exerted by the soil surfaces that bacterial cells are exposed to (MacDonald and Duniway, 1978). As a result, in order to translocate in soil, bacterial cells have to resist the attractive forces exerted by soil surfaces. Additionally, soil density (linked to soil compaction) is an important factor that influences bacterial motility in soil. That is, higher soil bulk densities clearly limit the movement of bacterial cells with percolating water (van Elsas et al., 1991).

2.3 Types of bacterial movement through soil

2.3.1 Active movement

In the presence of water that increases the connectivity across soil micro-aggregates, bacterial cells can move actively via flagellar motility, in addition to the effects of Brownian movement (Griffin and Quail, 1968). Flagellar motility often overrules this phenomenon (Abu-Ashour et al., 1994). For instance, flagellar motility can direct movement towards or against chemical gradients (e.g. of nutrients) — that is, chemotaxis. In an early experiment, it was found that *Azospirillum brasilense* and *Pseudomonas fluorescens* move in soil (up to 80 mm) towards wheat seedlings and synthetic attractants, thus categorizing a directional attraction of these bacteria which influence plant growth (Bashan, 1986). Conversely, one recent study (in which chemotaxis was not induced) showed that *Bradyrhizobium japonicum* was able to move up to 7 mm away from its inoculation site in water-saturated soil via flagellar activity (Covelli et al., 2013). Interestingly, the formation of multicellular structures by bacteria can support their migration in soil. One example is given by *Bacillus cereus* (strain ATCC14597). In fact, growing cells can switch to a multicellular phenotype, forming chains and then clumps. These chains and clumps span a
considerable distance in soil (ca. tens of micrometers), thus resulting in the movement of the bacteria that overcome structural gaps in the soil matrix (Vilain et al., 2006). This sliding translocation, as explained, is driven by the elongation and division of cell in chains (Henrichsen, 1972; Vilain et al., 2006), rather than via active flagellar motion.

2.3.2 Passive movement

Passive movement in soil can occur due to water flow, wind blows or through other organismal vectors. First, water flow through the soil pores can act as an important mechanism for bacterial movement. In several experiments, percolating water has been found to facilitate large-scale/-distance (up to 400 mm) bacterial translocation in soil (Breitenbeck et al., 1988). The rate of translocation is determined by parameters of the soil pore network, the water flow rate and the number of times the soil is flushed (Trevors et al., 1990). Moreover, the contextual soil properties, the initial moisture content and the soil bulk density are also key aspects that drive translocation (van Elsas et al., 1991). In nature, rain fall and irrigation clearly provide water flow through soil.

The force of wind blowing over the soil surface also constitutes a driver of microbial translocation over larger distances (Kellogg and Griffin, 2006). Microbial cells can be picked up by wind directly and transported over distances up to 1 km. Alternatively, cells attached to particular soil dust (which is lifted high by the wind) can disperse over even longer distances, reaching up to 5000 km (Kellogg and Griffin, 2006). However, only those bacteria that survive under harsh atmospheric conditions (UV radiation, desiccation and freezing within clouds) were found to be detectable at the destination (Kellogg and Griffin, 2006; Peter et al., 2014). Spore-forming bacteria (such as Bacillus species) represent one of the most abundant bacterial species that survive such long-distance dispersal by wind (Prospero et al., 2005). Also, the success of long-distance dispersal depends on the successful establishment of a new population in the recipient environment. As such, it depends on local conditions, such as the presence of a resident community and harshness from e.g. freezing snow (Meola et al., 2015).
2.3.3 Soil organisms mediating movement

The movement of bacterial cells in soil can be driven externally, i.e. bacterial cells can “hitchhike” along with other (moving or growing) soil organisms. This hitchhiking effect constitutes a mechanism that has been relatively understudied. For example, earthworms, nematodes, protozoa and soil fungi are major vectors that spur the dispersal of associated bacteria, acting either as “trucks” (earthworms, nematodes, protozoa) or as “networks” (fungi and plant roots). Hereunder, we briefly examine the impact of these two modes of bacterial translocation and their implications for bacterial cell dispersal.

2.3.3.1 Trucks

Nematodes, earthworms, protozoa, next to other soil animals (including soil arthropods), can be regarded as “trucks” that move through the soil, i.e. these organisms constitute individual transport units that can take on bacterial cargo, thus leading to a dispersal trajectory of the microbial cell along with the “truck” path. Bacteria can hitchhike along with such trucks by adhering to the available surfaces (Hekman et al., 1995; Daane et al., 1996). Extensive studies have shown that nematode/earthworm intestines represent complex systems that enable “truck-like” bacterial spread through soil. In the absence of earthworms, Pseudomonas fluorescens C5t (pJP4) showed limited dispersal through soil. On the other hand, the presence of the earthworms Lumbricus terrestris, Lumbricus rubellus and Aporrhectodia trapezoides moved bacteria to deeper soil parts, in dependency of the depth the earthworm could reach (Daane et al., 1996). Further studies found that earthworm guts harbor microorganisms that are acquired from the soil and subsequently selected by the host (Byzov et al., 2007; Singh et al., 2015). Thus, the natural movement of earthworms promotes the dispersal of particularly selected bacterial types. Here, uptake, survival and release are the three key steps that determine the successful dispersal of bacterial cells along with the moving earthworm. In a similar way, soil nematodes (Berg et al., 2016) and arthropods also contain specialized microbiomes that are shaped by similar processes, with fundamental implications for the modulation of the soil microbiome dispersal (Thimm et al., 1998; Agamennnone et al., 2015). Moreover, once these carrier organisms die, a
decomposition process ensues and specialized bacterial cells increase in abundance in the surrounding soil. Thus a hotspot for decomposer bacterial outgrowth can take place, fostering the “net” movement within the soil.

### 2.3.3.2 Networks

Plant roots and fungal hyphae both form structures that constitute interconnected networks of biological surfaces in soil. Such connected surfaces are colonized by microbes and constitute excellent “highways” along which bacterial cells may be able to move.

**Plant root networks** A classical example from an early study showed that, mediated by type I and type III fimbriae, Klebsiella strains effectively attach to root cap cells and to the root elongation zone, thus assisting the bacterial migration along with the growing roots (Haahtela et al., 1986). Similarly, Kamilova and colleagues showed that several bacteria, in association with tomato roots, can act as biocontrol agents and take profit from extension of the roots to migrate. In this particular case, all bacterial strains were motile, showing higher colonization ability on the root than the competitive tomato root tip colonizer Pseudomonas fluorescens WCS365 (Kamilova et al., 2005). Furthermore, Rhizobium radiobacter F4 first colonized the primary roots (1 cm in length) of barley at 5 days post-inoculation (5 dpi), then, it spread to secondary root, and covered the primary and secondary roots subsequently at 30 dpi (Glaeser et al., 2016). It is clear that the ability to attach to biological surfaces is a key factor that fosters the successful bacterial colonization of root tips, thus, migration along with developing roots is facilitated, whereas dispersion and/or chemotaxis are crucial for microbial transport process on mature roots, where tissues are immobile (Dupuy and Silk, 2016).

**Hyphal networks** There is an increasing body of literature highlighting fungal growth as a mechanism promoting bacterial movement in soil (Kohlmeier et al., 2005; Warmink and van Elsas, 2009; Furuno et al., 2012; Yang et al., 2016). Kohlmeier et al. first clearly revealed the working and importance of a “fungal highway” as a transport network for particular bacteria through soil (Kohlmeier et al., 2005). This revealing finding actually was a reconfirmation of much earlier findings on fungi spurring the movement of bacteria through soil (Wong and Griffin, 1976). In our own
Bacterial dispersal in soil – a review

In our recent work, we found that flagella play a key role in this process, whereas the type three secretion system and type four pili have minor influence (Yang et al., 2016; 2017). Thus, fungal hyphae growing through the soil and forming mycelial networks are essential for the efficient motility of particular bacterial types. We further found that only soil-dwelling hyphae, which were hydrophilic, can support bacterial migration. In contrast, aerial hyphae, which are more hydrophobic, did not allow efficient migration (Vila et al., 2016). Based on our findings, and in line with findings by Furuno et al. (Furuno et al., 2010), we posit that a water film formed around the soil-borne fungal hyphae is critical to the occurrence of bacterial swimming motility driven co-migration along the fungal highway. For details on the mechanism by which bacterial cells migrate along with underground transportation networks, see Box 2.1.

2.4 An “underground transport web” that drives the migration of key bacteria through soil

In line with the concept of major bacterial spread through soil by “truck-like” or “network” vector organisms, we posit that both of these two transport systems can lead to strongly divergent ecological outcomes. Whereas the soil trucks, particularly earthworms, protozoa and nematodes, drive pulsed cargo
transport that results in stochastic and spatially idiosyncratic delivery, the
networks provided by plant roots and fungal hyphae constitute a connected
“underground transport web” that facilitates a rather continuous (and
homogeneous) bacterial dispersion along the defined biological highway.
Moreover, plant roots, fungal hyphae and soil animals may even have
contact with other transport vectors in soil, resulting in overall complex
patterns of bacterial dispersal (Figure 2.1).

**Box 2.1** Processes involved in bacterial cells moving along with
fungal hyphae in soil

Several models have been proposed for bacterial migration along with fungal
networks. Based on all studies, including those of our own group, we propose the
process of bacterial migration along with fungal hyphae to proceed in soil as
follows:

1. **approach**: soil-borne bacterial cells get closer to an emergent or existing fungal
   surface. This may occur because of fungal hyphal elongation, which places the
   fungal surface in close vicinity of the bacterial cells. Bacterial cells may also move
to fungal surfaces mediated by other soil organisms (such as other fungal hyphae,
plant roots or earthworms).

2. **attachment**: some (but not all) bacterial cells bind to the fungal surface and
   adhere to it. The key driver here is staying in contact with fungal nutrient-rich
   surface areas, such as found at the apical tip. In this step, type three secretion
   system and type four pili may improve the competitive ability.

3. **growth**: bacterial cells utilize nutrients secreted by fungal hyphae and
   reproduce on the fungal surface.

4. **spread**: spread (movement) might be driven by chemotaxis. Within-population
   competition is another factor of importance here. Along with bacterial population
   increases by growth, the locally available nutrient level decreases, and so
   competition between cells augments. Some cells may leave the origin microsite and
disperse along with the fungal hyphae. Sensing and moving to fungal exudates
   (which is termed chemotaxis) may play a role. In this step, the presence of a water
   film around fungal hyphae is critical.

5. **establishment and formation of new niche**: via active motility, bacterial cells
   reach new microsites at the fungal surface, and repeat the former steps 1-4.

6. **biofilm formation**: by repeating steps 1-5, eventually a large population of
   bacterial cells occupies the hyphal surface, forming a biofilm around it.
Figure 2.1 Underground transport web in soil that facilitates bacterial motility in soil. (a) earthworm “trucks”; (b) plant toot network; (c) fungal hyphal network; (d) earthworm surface (blue) or intestine (dark green) associated bacteria; (e) plant root associated bacteria; (f) fungal hypha associated bacteria; (g) soil organisms connected with each other and bacterial cells can move from one organism to another.
2.4.1 Trucks support pulsed cargo delivery

The earthworms and/or nematodes that constitute the “truck-like” cargo system in soil facilitate temporally- and spatially-explicit bacterial migration and delivery. Depending on their typical life modus, the organisms (e.g. some specific earthworms) are known to either move up/down, horizontally or erratically in soil. Upon transporting bacterial cargo, they haphazardly release cells, or do so along with casts. In their behavior in soil, they have ample opportunities to interact with plant roots or fungal hyphae, which may result on either idiosyncratic or organized patterns of bacterial dispersal (Figure 2.1). For instance, one key outcome of such “truck-like” transport was found to be the movement of *Sinorhizobium meliloti* from bulk soil to the roots of the legume *Medicago truncatula* by the soil nematode *Caenorhabditis elegans*. The organism was stably carried by the truck which was attracted to the plant roots (by plant-released volatiles) where it was shown to unload its bacterial cargo (Horiuchi et al., 2005).

2.4.2 Networks yield highways for continuous cargo delivery

We convincingly revealed that *P. terrae* BS001 is able to translocate along with several fungal species, including saprotroph strain *Lyophyllum* sp. strain Karsten, phytopathogen strains *Rhizoctonia solani* AG3 and *Fusarium oxysporum* Fo47, biocontrol strain *Trichoderma asperellum* 302 (Nazir et al., 2014). The network formed by these fungi in soil can facilitate the spread of BS001 continuously on a large scale. On top of such fungal-driven spread, connections with other fungal hyphae and/or plant roots exist. For instance, common mycorrhizal network play a critical role in long-distance transport of nutrients through soil ecosystem (Bücking et al., 2016). Thus, a picture emerges of an often large (semi)continuous fungal-mediated connected belowground network, along which specific bacteria are able to migrate in accordance with local factors affecting this migration. Events of bacterial biofilm formation and disruption, growth and death and bacterial movement are thought to determine the resulting fungal-driven dispersal patterns.
### 2.5 Ecological effects of bacterial dispersal through soil

The nutrients (sources of carbon and energy) that support bacteria in soil are generally distributed in a discontinuous way. In most soils, several hotspots of enhanced nutritional status exist. In these, a suite of energy-rich sources of nutrients is, although often ephemeral, available. Such hotspots can be found in specific soil sites as the mycosphere, the mycorrhizosphere, the rhizosphere and the detritusphere (Kuzyakov and Blagodatskaya, 2015). By moving in the soil matrix, bacterial cells are able to explore newly-available soil area and thus utilize the locally available nutrients from which they had previously been separated. Thus, the presence of either or both of an underground transportation network and a “truck delivery” systems are key assets that greatly foster the net bacterial dispersal in soil.

Bacterial dispersal may be a *conditio-sine-qua-non* for efficient remediation of polluted soil that needs bacterially-mediated degradation activity. A key early finding was that fungi (Kohlmeier et al., 2005; Furuno et al., 2012) and/or earthworms (Li et al., 2015) are able to mediate the dispersal of pollutant-degrading bacteria to key sites in soil, allowing them to degrade toxic compounds. Here, one should take into account that also non-single-strain migrator bacteria may move along with fungal hyphae, by a hitchhiking effect (Warmink et al., 2011). On another notice, some human pathogens were found to also disperse in soil, reaching vegetable crops, which is an undesirable process that may subsequently threaten human health (Williams et al., 2006).

With respect to mutual effects, some fungal-associated bacteria can exert beneficial effects to the host. For instance, *P. terrae* BS001 was found to protect the fungal host, *Lyophyllum* sp. strain Karsten, against the antifungal agent cycloheximide and antifungal bacterial strain *P. fluorescens* CHA0 (Nazir et al., 2014). Similarly, the hypermotile *P. fluorescens* F113 strain showed higher competitive colonization on the alfalfa root surface than other strains, thus replaced other strains at root surface and improved biocontrol activity (Barahona et al., 2011). Moreover, plant growth promoting rhizobacteria can enhance plant growth (Pii et al., 2015) and gut-associated bacteria have been reported to provide essential amino acids for earthworms (Larsen et al., 2016). Dispersal extends the living area of these bacteria, and enhances the beneficial effects exerted on their hosts. Thus, the interactions
between bacterial cells and their hosts can be regarded as mutualistic if dispersal facilitation is included in our considerations.

### 2.6 Conclusions

Bacterial dispersal through soil occurs in diverse ways and is affected by several environmental factors. A key emergent concept is that of an underground transport network, in which both continuous transport (plant root and fungal networks) and pulsed transport ("truck-like" agents) are taking place. Dispersal is key to applications in environmental management, such as in polluted soil. Use of bacterial-dispersal-enhancing soil organisms, combined with pollutant degrading bacteria, may improve the degradation efficiency. Also, the use of combined biological agents can facilitate bacterial cells in colonizing roots and exert beneficial effects there. Additionally, bacterial dispersal may play an important role in the spread of phytopathogens between plant roots. Thus, it is critical to avoid dispersal via these avenues, along with combat of the pathogen. Moreover, human pathogen can be carried by moving water, to transfer from soil surface to underground water, or by wind to disperse to other area. This brings a huge risk in spreading of epidemic disease. Thus, bacterial dispersal in soil is an important environmental issue and more efforts should be paid in this area to understand the underlying mechanisms, which are likely species-specific.

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References


Nazir, R., Zhang, M.Z., de Boer, W., and van Elsas, J.D. (2012) The capacity to comigrate with *Lyophyllum* sp. strain Karsten through different soils is spread among several phylogenetic groups within the genus *Burkholderia*. *Soil Biology & Biochemistry* **50**: 221-233.


Van Elsas, J.D., Trevors, J.T., and van Overbeek, L.S. (1991) Influence of soil properties on the vertical movement of genetically-marked *Pseudomonas*


Yang, P., Zhang, M., and van Elsas, J.D. (2017) Role of flagella and type four pili in the co-migration of *Burkholderia terrae* BS001 with fungal hyphae through soil. *Scientific Reports* 7: 2997.