Microbial hitchhiking: how *Streptomyces* spores are transported by motile soil bacteria

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Abstract

Streptomycetes are sessile bacteria that produce metabolites that impact the behavior of microbial communities. Emerging studies have demonstrated that *Streptomyces* spores are distributed through various mechanisms, but it remains unclear how spores are transported to their preferred microenvironments, such as plant roots. Here, we show that *Streptomyces* spores are capable of utilizing the motility machinery of other soil bacteria. Motility assays and microscopy studies reveal that *Streptomyces* spores are transported to plant tissues by interacting directly with the flagella of both gram-positive and gram-negative bacteria. Genetics experiments demonstrate that this form of motility is facilitated by structural proteins on the spore coat. These results demonstrate that nonmotile bacteria are capable of utilizing the motility machinery of other microbes to complete necessary stages of their lifecycle.

Introduction

Bacteria belonging to the genus *Streptomyces* are an integral component of diverse ecosystems and are well-known to produce chemically diverse metabolites, including the vast majority of all clinically relevant antibiotics [1, 2]. Soil-dwelling Streptomycetes, such as *Streptomyces coelicolor* (Sc), colonize plant roots and provide the associated plant protection from potential phytopathogens through antibiotic secretion [1]. The symbiosis of Streptomycetes with their plant hosts has been shown to improve plant health and productivity, and thereby provides a potential sustainable solution to increase crop yields [2–5]. The lifecycle of Streptomycetes is complex and involves stages of aerial hyphae formation on the soil surface to produce spores, and spore germination on plant roots to produce filamentous colonies [1]. Immotile *Streptomyces* bacteria distribute their spores over long distances through attachment to insects and nematodes, but it is unclear how they relocate over short distances to their preferred microenvironments such as plant root systems [6, 7].

While *Streptomyces* are nonmotile, many other soil microbes, such as *Bacillus subtilis* (Bs) and *Pseudomonas fluorescens* (Pf), are motile and can move through their environment by regulating the rotation of flagella [8]. On solid surfaces, flagellar rotation enables cell swarming. During swarming, cells are densely packed and continuously move outward toward unoccupied areas. In liquid media, flagella enable cell swimming where the cells move independently and can rapidly change swimming direction. In addition to flagellar motility, some bacteria can also move through a passive diffusion on surfaces called sliding [8]. Sliding does not involve flagella but occurs when cells are pushed through the forces generated by the outward growing colony.

Recent reports have revealed that microbe transport by inter-species interactions can occur between motile and immotile microbes [9]. These studies demonstrate that inter-microbial transport occurs among organisms natively found on abiotic surfaces [10, 11], plant surfaces [12], and within the soil [13, 14]. In some instances, the transportation of human pathogens are facilitated on abiotic surfaces, including nonmotile Staphylococcal species that directly adhere to their mobile partners [10]. *Aspergillus fumigatus* (Af) spores that interact with the flagella of motile bacteria...
[14], various nonmotile human microbiome bacteria that are
carried by Capnocytophaga gingivalis [15], and Legionella
pneumophila that is transported internally by their amoebae
hosts [16].

Here, we demonstrate that spores of the sessile Streptomyces,
such as Sc, are transported by Bs to their preferred microenvironment. Sc and Bs are both soil-dwelling bacteria that utilize plant root exudate as a nutritive source [1, 17]. Using microscopy methods, motility assays, and genetics approaches, we demonstrate that Bs transports Sc spores via direct attachment to Bs flagella, a mode of transportation we call “hitchhiking”. Hitchhiking is dependent on the conserved rodlin
proteins, which form a fibrous outer layer on the spore
cell coat of almost all Streptomyces, but with a hitherto
unclear function [18, 19]. These results exemplify that
nonmotile bacteria are capable of utilizing the motility
machinery of other microbes to occupy advantageous
environments, and that this mode of transport may be
widespread in nature.

Results

Bacillus subtilis disperses Streptomyces coelicolor
spores

Transportation of Sc spores by Bs was demonstrated by
mixing isolated Sc spores with a liquid culture of Bs
followed by inoculation onto an agar swarm plate and
incubation. After 5 days, Sc colonies are visible on the
plate and are only dispersed in the presence of Bs in all
samples tested \((n = 13)\) (Fig. 1A). Spore dispersal by Bs
occurs across the entire surface of the plate and to the
plate’s edge \((4.5 \text{ cm from the inoculation point})\). To
demonstrate that the Sc spores are being moved by Bs
cells and not merely “floating” in the expanding Bs col-

dony, we conducted identical assays but inoculated the Sc
spores and Bs culture separately and onto different areas
of the plate. The resulting Sc colonies form streaks across
the plate that emanate from the Bs inoculation site in a
predictable manner in all samples tested \((n = 10)\)
(Fig. 1B). This experiment was repeated on 12 cm plates
and demonstrate that Sc spores are dispersed to the edge
of the plate, which is 10 cm from the spore inoculation
point \((n = 3)\) (Fig. S1A). To determine the efficiency
of transport, this experiment was conducted with dilutions of
Sc spores so that the total number of Sc colonies would
germinate without overlap and could be individually

counted. The results demonstrate that \(86 \pm 5.6\%\) of
the apparent colonies are located outside of the initial
inoculation point under these conditions \((n = 4)\)
(Fig. S1B).

B. subtilis transports S. coelicolor spores to plant
tissues

In nature, Sc and Bs thrive near plant roots that excrete
exudates but only Bs can move toward the root systems. We
conducted assays to determine if Bs can transport Sc spores
to plant tissues. Assays with the Bs strain alone demon-
strate that the plates become “cloudy” with Bs cells in areas
around plant tissues, perhaps due to the presence of nutritive
plant exudates that facilitates bacterial growth (Fig. 1C).
Like previous experiments, the Sc spores alone do not
exhibit movement unless they are co-inoculated with Bs
cells, and the dispersed spores preferentially establish
colonies near plant tissues in all instances \((n = 5)\) (Fig. 1C).

Spore dispersal occurs with swarming and sliding Bs
strains

Bs has two modes of flagellar-mediated motility, swimming
and swarming, that occur in liquid environments and on
solid surfaces, respectively. When Bs senses that it is on a
solid surface, it will differentiate into a swarmer cell that has
a significant increase in the number of flagella and produces
hydrophobic surfactants, such as surfactin, to efficiently
spread across the surface [20–22]. In our experiments, we
utilized an undomesticated strain of *Bt* (NCIB3610) that can swarm, unlike common laboratory strains that lack the ability to differentiate into swarmer cells and fail to produce surfactin to undergo swarming motility (Table S1) [20, 22–24]. To determine if the *Sc* spores are transported by both swarming and swimming motilities, we repeated the experiments with a laboratory-cultivated *Bt* strain that is incapable of swarming, and spore transport does not occur on the agar swimming plates (0.27% agar) (Figs. 2A and S2) [20]. Importantly, the laboratory-cultivated strain has accumulated many genetic defects in addition to defective swarming capabilities. To ensure that the decrease in spore dispersal can be attributed to limitations of bacterial swimming, we utilized a *Bt* ΔmotAB strain with an undomesticated genetic background but has been genetically altered so that it is only capable of swimming motilities (ΔepsH srfAA, DK1484). Like the laboratory-cultivated strain, this strain does not disperse *Sc* spores on swim plates (Figs. 2A and S2). Therefore, we conclude that spore transport can be accomplished by swarming motility but not swimming motility.

In addition to flagellar motilities, *Bt* can also move through sliding on surfaces. To determine if spores can be dispersed through sliding, we utilized a *Bt* mutant that has flagella but the flagella cannot rotate (ΔmotAB, DS222) [25], and a mutant that does not possess flagella (Δhag, DS1677) [26]. The ΔmotAB strain moves spores over the entire surface and to the edge of the agar plates in all samples (4.5 cm from the inoculation point) (n = 6) (Figs. 2B and S2). The number of dispersed spores cannot be ascertained due to overlapping colonies that result in apparent smears across the plates, but each plate contains over 100 dispersed *Sc* colonies. The Δhag strain shows severely reduced dispersal, where an average of 3.2 spores are transported with an average maximum distance of 0.95 ± 0.85 cm from the inoculation point (n = 6) (Figs. 2B, S2 and S3). Therefore, we surmise that spore dispersal can also occur through sliding but is facilitated by the presence of flagella (Fig. 2C). As an additional control, we also conducted these experiments by first spreading the *Bt* cells across the surface of the plate and then inoculating the *Sc* spores in the center. Spore dispersal was significantly reduced for both the *Bt* WT (2.06 ± 0.05 cm) and ΔmotAB (1.25 ± 0.16 cm) strains when compared to co-inoculation in the center of the plate (Figs. S2 and S4). These data indicate that the *Sc* spores are transported by *Bt* cells that continuously move away from the inoculation point and are not transported after the motile *Bt* has covered the surface of the plate.

**S. coelicolor** spores attach to *B. subtilis* flagella

We utilized several microscopy methods to elucidate a mechanism for *Sc* spore dispersal by *Bt*. Fluorescently
labeled Sc spores were imaged under a fluorescence microscope and were immotile as expected (Supplementary Movie 1). However, when Bs cells were added to the fluorescent spores, the spores localize near the Bs cell poles (Supplementary Movie 2 and Fig. 3A). In some instances, the spores are stationary on the surface of the glass slide and an associated Bs cell is seen rotating around the spore (Movie 1). The observed Bs cell rotation in these assays is reminiscent of rotations seen in Bs cells that have their flagella chemically tethered to a solid surface, whereby the torque generated by the immobilized flagella induces rotation of the cell body [27]. This observation suggests that the Sc spores adhere directly to the Bs flagella, and therefore effectively mimic a flagellar tether in these instances. To verify that the Sc spores do not directly interact with the Bs cell body, we imaged a mixture of Sc spores and Bs cells with a cryo-electron microscope. Like the fluorescence microscopy images, the Sc spores are localized near the Bs cell poles but do not make direct contact with the Bs cell body (Figs. 3B and S5A). In total, \(~77\)\% of the spores are located within 1 \(\mu m\) of a Bs cell pole \((n = 35\) spores). To determine if the spores interact with Bs flagella, we utilized a Bs “mini-cell” strain \((minD::TnYLB)\) that lacks the excreted material inhibiting their direct visualization. Indeed, when mixed with Sc spores, the flagella can be seen co-localizing with the spores in two-dimensional cryo-EM images (Figs. 3C and S5B).

Sc spore adherence to Bs flagella was further confirmed by visualization of a mixture of Sc spores with sheared flagella via fluorescence microscopy. Fluorescent labeling of Bs flagella was accomplished using a Bs strain (DS1919) that is mutated in a single flagellin residue (T209C) [28]. The surface-exposed thiol allows for direct labeling with dyes that possess a reactive maleimide group. This dye also labels proteins on the surface of the Sc spore coat. Indeed, when sheared Bs flagella are isolated and mixed with spores, and unassociated flagella are washed from the mixture, a majority of spores still retain associated flagella. In total, \(~64\)\% of the spores are associated with flagella \((n = 130\) spores). In some instances, large “clumps” of spores entangled in flagella are observed (Figs. 3D and S5C). In comparison, the flagella without spores added do not form aggregates and are randomly dispersed (Fig. S5C).

Hitchhiking is conserved in Streptomyces

To determine if spore dispersal by Bs also occurs in other \(Streptomyces\) species, we conducted Bs swarm plate assays with \(Streptomyces tendae\) (St), \(Streptomyces griseus\) (Sg), \(Streptomyces scabies\) (Ss), and \(Streptomyces avermitilis\) (Sa). To quantify spore dispersal, we prepared swarm plate assays where Bs cells were inoculated at the center of the plate (9 cm in diameter) and the isolated spores are inoculated in four equidistant positions around the Bs inoculation site. The maximum dispersal distance, which is the distance from the center of the spore inoculation site to the most distant dispersed colony, was measured for each of the four spore samples. The wild-type (WT) Sc spores are dispersed by Bs in 100\% of samples and are moved an average maximum distance of \(2.67 \pm 0.43\) cm from the initial inoculation point \((n = 20)\). Likewise, these assays demonstrate that St \((n = 12)\), Sg \((n = 8)\), and Ss \((n = 8)\) spores are dispersed in 100\% of samples to similar distances as WT Sc spores. However, the Sa spores are dispersed at significantly shorter distances \((n = 12)\) (Fig. 4A, B) and are dispersed in 83\% of the samples. As the last common ancestor of Sc and Sg existed more than 200 million years ago and both species are capable of hitchhiking, these data suggest that the ancestor also possessed this dispersal mechanism and it remained conserved.

Spore dispersal by \(B. subtilis\) is facilitated by the rodlin proteins

The outer surface of most \(Streptomyces\) spores is characterized by a fibrillar rodlet layer, which is a striated pattern of pairwise aligned rodlets composed of the rodlin proteins [18, 19]. Scanning electron microscopy (SEM)
Fig. 4 Hitchhiking of *Streptomyces* spores is facilitated by the presence of rodlin proteins. A Bs swarm assays with both wild-type (WT) *Streptomyces* spores (*S. coelicolor* *n* = 20, *S. tendae* *n* = 12, *S. griseus* *n* = 8, *S. scabies* *n* = 8, *S. avermitilis* *n* = 12) and Sc spores lacking the rodlin proteins (*Sc ΔrdlAB* *n* = 24) demonstrate that spores are dispersed in all tested WT species but with *S. avermitilis* dispersed the shortest distance, and dispersal is abrogated by the loss of the rodlins in Sc. Results are expressed as the mean of dispersal distance ± standard error of the mean. Differences for *Sc ΔrdlAB* and *Sa* dispersal compared to WT *Sc, St, Sg,* and *Ss* strains are statistically significant using a two-tailed null hypothesis significance test

(p < 0.05). B Representative images of *Bs* spore swarm plates from A. The *Bs* inoculation site is denoted with an asterisk. C SEM of WT *Sc* spores shows the rodlet layer with pairwise rodllets of 20 nm spacing. D A representative cryo-ET image of isolated *Sc* WT spores shows that the rodlet layer does not cover the entire spore but leaves the poles exposed (*n* = 22). E Cryo-ET reconstructions show that flagella preferentially interact with the rodlet layer (*n* = 12). Scale bar 100 nm. F Segmentation of the reconstruction from E clearly demonstrates the flagellaroldin interaction. Spore body: purple, flagella: yellow. Scale bar 100 nm (Color figure online).

images of *Sc* and *Ss* spores show the striated rodlet layer (Fig. 4C). In previous studies, the rodllets of *Sc, S. lividans, St, Sg,* and *Ss* were visually indistinguishable [19, 29]. Using electron microscopy images from this and previous studies, we measured the spacing of the rodllets in these species, which is highly conserved and around ~20 nm (when measured from the center of the rodlet fibers) (Table S2). Furthermore, the rodlin proteins from *Sc, St,* and *Sg* have ~34% sequence identity despite the species’ distant evolutionary relation (Fig. S6) [19].

Intriguingly, *Sa* spores are less widely dispersed than the other *Streptomyces* species and it is the only tested species that natively lacks rodlin proteins [19]. In agreement, an *Sc* mutant strain that lacks the rodlin proteins (*ΔrdlAB*) abrogates hitchhiking by *Bs* (*n* = 3, Figs. S2 and S7). Importantly, previous studies demonstrate that the *Sc ΔrdlAB* strain is not delayed in germination, and does not exhibit any behavioral or phenotypic change compared to the WT strain with the exception of the rodllet layer [18, 19, 30]. In contrast, *Sc* mutants that lack proteins which produce polysaccharides found on the surface of *Streptomyces* (*ΔcslA* and *ΔmatAB*) are unaffected (*n* = 3, Figs. S2 and S7) [31]. However, spore dispersal was not completely abolished in the *ΔrdlAB* strain. Using identical swarm plate assays described in the section above, the *ΔrdlAB* strain is dispersed with an average maximum distance of 0.46 ± 0.82 cm from the initial inoculation point and dispersal occurs in 33% of the samples (*n* = 24) (Fig. 2).

To characterize how rodlins interact with flagella in three dimensions, we conducted cryo-ET experiments of samples containing *Bs* minicells and *Sc* spores. Reconstructions show that the *Sc* spores are oval shaped and possess a thick coat. The rodllet layer can be seen as a sheath around the lateral sides of the spore with frayed edges, leaving the poles exposed, and suggest that the rodllet sheath easily peels away from the cell body (*n* = 22) (Fig. 4D). A similar spore morphology has also been observed in *Streptomyces albicus* [32]. *Bs* flagella accumulate around and directly interact with the rodllet layer (*n* = 12) (Fig. 4E, F and Movie 2). However, due to the thickness of the spores the resolution is limited and we could not deduce if the flagella preferentially bind specific features of the rodllet layer. Collectively, these data suggest that the rodllet layer facilitates spore dispersal by interacting directly with flagella.

**Hitchhiking of *S. coelicolor* spores is not limited to *Bacillus***

Although *B. subtilis* is ubiquitous in soil, other genera are also flagellated and may also contribute to dispersal of *Sc*
Spores. We therefore conducted swarm plate assays with a P. fluorescens WT strain (R1SS101), which is also natively associated with plant roots [33]. Importantly, Pf disperses spore similarly to Bs, but Sc colonies appear in patterns that are reminiscent of Pseudomonas swarm patterns [34] (n = 6) (Fig. S8). These data demonstrate that hitchhiking is a widespread mechanism that allows Streptomyces spores to disperse at cm-scales (Fig. 5).

Discussion

Sessile Streptomycetes have a complex lifecycle that involves formation of aerial hyphae that differentiate into spores. The spores of some Streptomyces species, including Sc, are dispersed over long distances by direct attachment to insects and nematodes [6]. Intriguingly, recent reports identify that specific volatile metabolites secreted by Streptomycetes attract arthropods as a mechanism for spore dispersal [7], and they can induce the formation of Streptomyces “explorer cells”, while simultaneously starving microbial competitors [35]. However, it is unclear how the spores are dispersed specifically at the centimeter scale to plant root micro-environments. Here, we demonstrate that Streptomyces spores are able to utilize the motility machinery of motile soil microbes by directly attaching to their flagella. While these experiments demonstrate that Sc spores are dispersed by Bs and Pf regardless of their destination, these motile bacteria are also known to associate with plant roots. Therefore, this mechanism of dispersal, called hitchhiking, may provide Streptomyces spores a mechanism for translocation to beneficial environments. Indeed, assays with A. thaliana plants demonstrate that Bs can transport Sc spores to plant tissues. This allows spores to germinate near nutrient-rich plant exudate to generate filamentous colonies that produce antibiotics, thereby protecting the plant from potential phytopathogens.

Hitchhiking is facilitated by two spore coat proteins, RdlA and RdB, which are conserved in most Streptomycetes. These proteins assemble into pairwise aligned filaments, called rodlets, on the outer surface of the spores and are spaced ~20 nm apart (Table S2). Until now, the function of the rodlets has remained elusive [30]. Interestingly, the diameter of the bacterial flagellar filament is also ~20 nm [22, 36]. Therefore, it is possible that the rodlet layer provides a gripped surface for the flagella, which become “wrapped” in the grooves made by the rodlin proteins and thereby facilitates spore transport. However, our cryo-ET data can not support such speculations due to the limited resolution of the whole-cell reconstructions. Therefore, it is still unclear what properties of the rodlet layer encourage interactions with flagella.

Emerging studies have demonstrated that flagella preferentially interact with hydrophobic surfaces and flagellin can undergo methylation to increase flagella hydrophobicity [37–39]. This increase in hydrophobicity allows pathogenic bacteria to adhere to host cells [37–39], and flagellar adherence to plant cells has also been implicated in establishing colonization [40, 41]. Flagella hydrophobicity may facilitate interactions with hydrophobic spores and may account for spore transport that is seen in the absence of rodlin, given that the spore surface without rodlin remains hydrophobic (in Sa WT and Sc ΔrdlAB strains) [18, 42, 43]. Therefore, the same flagellar interactions that facilitate adherence to plant roots may also contribute to adherence to spores, and necessitate that motile bacteria not evolve to eliminate this interaction. Since spore:flagella interactions are in-part facilitated by hydrophobic interactions, this mode of transport may be influenced by environmental factors that impact Van der Waals screening distances such as salt concentrations and pH.

Although spore hitchhiking seems disadvantageous to the motile partner, previous research has shown that fungal hyphae can form so-called “fungal highways” that serve as bridges for motile bacteria over air gaps (such as air gaps found in soil) [14, 44, 45]. Like fungi, Streptomycetes form...
aerial hyphae that are structurally similar to fungal hyphae [46]. Therefore, Streptomyces may be able to form “bacterial bridges” for their motile partner, but such interactions have not been reported yet. If such “bacterial bridges” do form, this would supply the system with a synergistic transport that has been previously observed between nonmotile fungal spores and motile bacteria [14].

The hitchhiking model is supported by a previous study that examines the interaction of two other root-colonizing microbes: the immotile fungus Af and the motile bacterium Paenibacillus vortex (Pv) [14]. Af spores are demonstrated to be dispersed by Pv in a swarming-dependent manner via direct attachment to flagella; dispersal is abrogated by the addition of excess purified Pv flagella or perturbations to the Af spore coat [14]. Furthermore, scanning EM micrographs show direct contact between Pv flagella and Af spores [14]. Although this study does not identify the spore coat component(s) responsible for adherence to flagella, Aspergillus spores also possess a rodlet layer [47]. Additionally, this study demonstrates that some Penicillium species are also transported by Pv, and these fungi possess a rodlet layer [48]. Collectively, these data may suggest that hitchhiking of spores onto motile bacteria via the formation of a striated rodlet layer is a dispersal mechanism that convergently evolved in both domains of life.

The colonization of plant roots by some Streptomyces improves plant health and performance in a natural and sustainable manner [2–5]. Therefore, our data are applicable to industrial initiatives that aim to improve soil conditions for Streptomyces root colonization. Likewise, many Aspergillus fungi, like Af and A. niger, are human and plant pathogens. Therefore, insights into hitchhiking of these sessile organisms may elucidate unknown infection mechanisms.

**Methods and materials**

**Streptomyces spore isolation**

The following Streptomyces strains were used in this study: S. coelicolor M145 [49], S. coelicolor ΔrdlAB6 [42], S. tenuae Tü9018c [50], S. griseus (ATCC 13273), S. avermitilis (ATCC 31267), and S. scabies ISP5078. Spores were harvested from MS agar plates and quantified as described before [49].

**Bacillus subtilis cultivation**

The undomesticated Bs strain NCIB3610 [23] 25% glycerol stock was placed into 5 ml of LB and grown overnight at 30 °C. After 16 h of growth, 100 μl of the overnight culture was diluted into 5 ml of LB and grown at 37 °C to an O.D. of 0.4–0.5.

**Pseudomonas fluorescens cultivation**

The Pf strain R1SS101 25% glycerol stock was placed into 5 ml of 50% TB and grown overnight at 30 °C. After 16 h of growth, 100 μl of the overnight culture was diluted into 5 ml of 50% TB and grown at 37 °C to an O.D. of 0.4–0.5.

**Swarm and swim plate assays**

Swarm and swim plates were conducted on nutrient broth plates (0.5% peptone, 0.3% yeast extract, 0.5% NaCl) containing specific amounts of agar (0.27–0.5%). All components were mixed, autoclaved for 20 min, and 30 ml of the media was poured into a plastic petri dish with a 9 cm diameter. The plates were cooled for 30 min in a sterile fume hood and then stored in a 4 °C fridge for a maximum of 1 week. To determine if Sc spores are dispersed by Bs, 3 μl of Bs cells are inoculated onto the plate and 3 μl of Sc spore stocks are either added on the Bs inoculation site or to a separate inoculation site. The plates are incubated at 30 °C for 5 days and imaged on a light box.

For all plate images, the distance of spore dispersal was determined using Image J bundled with Java 1.8.0_172 software. To measure the distances, the diameter of the petri dish (9 cm) was used as a reference scale. Then, a line was drawn from the inoculation point of the spores to the Streptomyces colony that is furthest from the inoculation point. The distance of the line was calculated based on the value of the reference scale.

**Fluorescence microscopy**

For fluorescent labeling of spores with unlabeled B. subtilis, 10 μl of Sc spore stock was added to 1 ml of iced LB. The 1 μl of the fluorescent styril dye, FM2–10 (Thermo Fisher Scientific), was added to the 1 ml solution and inverted to mix. Excess dye was removed by rinsing the spores 4X with 1 ml of iced LB via centrifugation and decanting. After the final decantation, 1 ml of Bs cells with an O.D. of 0.4 were added to the spores, mixed via pipetting, and incubated at ambient temperatures for 5 min. Immediately before imaging, 5 μl of the samples were placed on a glass slide and a glass coverslip was placed on top. The sample was imaged on a Zeiss Axiocscope A1 fluorescent microscope scope equipped with an Axiocam MrC5 camera (Zeiss) in the Institute of Biology Microscopy Unit using a GFP filter. Images were collected and processed using Axiovision software (Zeiss).
For fluorescent labeling of sheared *B. subtilis* flagella with spores, *B. subtilis* (strain Δhag amyE::Phag-hagT209C spec) was grown to an O.D. of 0.6, and 2 ml of the cells were pelleted via centrifugation and resuspended in 1 ml of PBS buffer pH 7.5. The 1 ml suspension was passed back-and-forth between two 5 ml syringes with 21 gauge needles that were connected by a plastic tube (10 cm long with an inner diameter of 0.58 mm). The cell suspension was gently passed back and forth between the syringes 50 times, with 1 min pauses every ten passes. The cells were removed from the mixture via centrifugation at 5000 × g for 5 min. The supernatant containing the flagella were then centrifuged once again to remove any residual cells. In total, 5 μg/ml Alexa Fluor 488 C₃ maleimide dye was added to the suspension and incubated for 5 min at room temperature. In total, 10 μl of *Sc* WT spore stock was then added to the mixture. The tube was gently inverted 50 times and the spores with associated flagella were pelleted via centrifugation at 5000 × g for 5 min. The pellet was washed with 1 ml PBS buffer pH 7.5 and centrifuged once again. The resulting pellet was resuspended in 50 μl PBS buffer pH 7.5. The sample was imaged on a Zeiss Axioscope A1 fluorescent microscope scope equipped with an Axiocam Mrc5 camera (Zeiss) in the Institute of Biology Microscopy Unit using a GFP filter. Images were collected and processed using Axiovision software (Zeiss).

**Cryo-electron microscopy**

*B. subtilis* cells were grown to an O.D. of 0.5 and 1 ml of *B. subtilis* cells were mixed with 5 μl *S. coelicolor* spores glycerol stock and incubated at ambient temperatures for 5 min. Cells were concentrated by centrifugation and 3 μl aliquots of the cell suspension are applied to glow-discharged R2/2 200 mesh copper Quantifoil grids (Quantifoil Micro Tools), the sample was pre-blotted for 30 s, and then blotted for 2 s. Grids were pre-blotted and blotted at 20 °C and at 95% humidity. The grids were plunge frozen in liquid ethane and stored in liquid nitrogen. The grids were imaged on a 120 kV Talos L120C cryo-electron microscope (Thermo Fisher Scientific) at the Netherlands Center for Electron Nanoscopy.

**Cryo-electron tomography**

*B. subtilis* mini-cell strain was grown from a 20% glycerol stock to an O.D. of 0.6 in 50 ml of LB. The cells were centrifuged at 8000 × g for 30 min. The supernatant was collected and then centrifuged at 12,000 × g for 20 min. The resulting cell pellet was resuspended in 20 μl of LB and 8 μl of WT *Sc* spore stock was added to the cell mixture. A 1/10 dilution of protein A- treated 10-nm colloidal gold solution (Cell Microscopy Core, Utrecht University, Utrecht, The Netherlands) was added to the mixture and mixed by pipetting. The grids were prepared using an automated Leica EM GP system (Leica Microsystems) with the sample chamber set at 20 °C and at 95% humidity. In total, 3 μl of the sample mixture was applied to a freshly glow-discharged copper R2/2 200 grid (Quantifoil Micro Tools), pre-blotted for 30 s, and then blotted for 2 s. The grid was plunge frozen in liquid ethane and stored in liquid nitrogen.

Images were recorded with a Gatan K3 Summit direct electron detector equipped with a Gatan GIF Quantum energy filter with a slit width of 20 eV. Images were taken at a magnification of ×19,500, which corresponds to a pixel size of 4.4 Å. Tilt series were collected using SerialEM with a bidirectional dose-symmetric tilt scheme (−60° to 60°, starting from 0°) with a 2° increment. The defocus was set to −12 μm and the cumulative exposure per tilt series was 160 e-/Å². Bead tracking-based tilt series alignment and drift correcting were done using IMOD [51] and CTFplotter was used for contrast transfer function determination and correction [52]. Tomograms were reconstructed using simultaneous iterative reconstruction with iteration set to 4. Segmentation was done in IMOD.

**Plant growth**

*Arabidopsis thaliana* Col-0 strain was grown from sterilized seedlings on sterilized plant MS agar media. Harvested *A. thaliana* seeds were sterilized in a sterile fume hood by incubation in 10% bleach for 30 min, washed with sterile water, and then incubated in 70% ethanol for 5 min. The seeds were then washed 6X with sterile water, placed on sterile filter paper, and placed in a dark 4 °C fridge for 3–4 days in a sterile and parafilm-sealed petri dish. Plant agar media plates were prepared by autoclaving Murashige and Skoog (MS) media (0.22% MS media with vitamins, 1.2% plant agar, 0.5% sucrose, pH 5.8) and pouring 100 ml of the media into square petri dishes with 12 cm length. The plates were allowed to cool for 1 h in a sterile fume hood. *A. thaliana* seeds were manually placed on the surface of the plates 1 cm apart by picking up the seeds with sterilized wooden picks. The plates were sealed with parafilm and placed in a climate-controlled plant growth chamber at a 20° angle so the plant roots grew on the surface of the media. The plant chamber was kept at 21 °C with a 16-h light cycle. The plants were allowed to grow for 1 month before use in chemoattraction assays (below).

**Chemotaxis attractant assays with plant roots**

Chemoattraction of *Bs* cells to plant roots in the presence and absence of *Sc* spores was conducted on minimal media
plates with 0.25% agar. The media was prepared according to previous methods [53] in round petri dishes with 9 cm diameter. One month old sterile A. thaliana plants were removed from their sterile media and placed on the edge of the minimal media plates. In total, 3 μl of B. subtilis culture was placed to the center of the plate, and then 3 μl of the isolated spore stock was also added to the center. Controls of each bacteria by itself were also prepared. The plates were incubated for 16 h at 30 °C and then placed in a climate-controlled plant growth chamber for 2 weeks. The plant chamber was kept at 21 °C with a 16-h light cycle. After Sc colonies were visible, the plates were imaged on a light box.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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