



HAL
open science

The root extracellular trap: A checkpoint controlling root tip accessibility to microorganisms

Alexia Gaudry, Magalie Bénard, Agnès Attard, Eric Nguema-Ona, Azeddine Driouich, Barbara Pawlak

► To cite this version:

Alexia Gaudry, Magalie Bénard, Agnès Attard, Eric Nguema-Ona, Azeddine Driouich, et al.. The root extracellular trap: A checkpoint controlling root tip accessibility to microorganisms. *Rhizosphere*, 2025, 33, 10.1016/j.rhisph.2025.101016 . hal-04917295

HAL Id: hal-04917295

<https://normandie-univ.hal.science/hal-04917295v1>

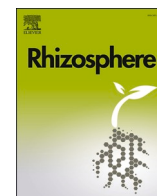
Submitted on 28 Jan 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License



The root extracellular trap: A checkpoint controlling root tip accessibility to microorganisms

Alexia Gaudry^{a,*}, Magalie Bénard^b, Agnès Attard^c, Eric Nguema-Ona^d, Azeddine Driouich^a, Barbara Pawlak^a

^a Univ Rouen Normandie, Normandie Univ, GLYCOMEV UR 4358, SFR Normandie Végétal FED 4277, F-76000, Rouen, France

^b Univ Rouen Normandie, Inserm, CNRS, Normandie Univ, HERACLES US 51 UAR 2026, PRIMACEN, F-76000, Rouen, France

^c INRAE, Université Côte d'Azur, CNRS, Institut Sophia Agrobiotech, Sophia Antipolis, F-06903, France

^d CMI-Roullier, Plant Nutrition Department, Crop Management Under Biotic and Abiotic Stress Group, F-35400, Saint Malo, France

ARTICLE INFO

Keywords:

Root secretions
Mucilage
Microbial motility
Rhizosphere
Rhizobacteria

ABSTRACT

The root tip and root-Associated Cap-Derived Cells (AC-DCs) release various organic compounds into the rhizosphere, forming the Root Extracellular Trap (RET), a network involved in root-soil microorganism interactions and root protection. This study investigates the role of soybean (*Glycine max*) and pea (*Pisum sativum*) RETs in interactions with soil rhizobacteria (*Bacillus subtilis* and *Pseudomonas fluorescens*) and the zoospores of the oomycete *Phytophthora parasitica* by examining their impact on microbial behavior. To this end, confrontation tests were performed, followed by imaging analyses of videos, in which the behavior of microorganisms (*i.e.*, swimming speeds and trajectories) was characterized and quantified. The results show that the RET alters microbial access to the root tip and modifies significantly speeds and trajectories. Inside the RET, the speeds of *B. subtilis* and *P. parasitica* zoospores decreased three and nine times respectively. Outside the RET, the speed of the bacterium remains unchanged, while that of the zoospores decreases twice. These findings highlight the influence of the RET on microbial movement and its importance in plant-microorganism interactions in the rhizosphere.

1. Introduction

The root is an underground organ that is essential for plant growth and development. Due to their position at the interface with the soil, roots, and particularly the root tip, play a fundamental role in the interactions of the plant with soil microorganisms (rhizosphere and root colonization, chemotaxis, nutrient acquisition) (Berendsen et al., 2012; Mendes et al., 2013; Chen and Liu, 2024). The root tip, encompassing the elongation and meristematic zones as well as the root cap, along with the cells detaching from the root cap known as root Associated, Cap-Derived Cells (AC-DCs) (Driouich et al., 2019), releases large amounts of high and low molecular weight organic compounds into the rhizosphere. The composition of these root secretions (*i.e.*, mucilage and root exudates) varies depending on endogenous or environmental factors, such as abiotic or biotic stresses (Hawes et al., 2002; Wen et al., 2007; Badri and Vivanco, 2009; Vives-Peris et al., 2020; Ropitiaux et al.,

2020). The assembly of the mucilage with the AC-DCs forms a complex network known as the Root Extracellular Trap (RET) (Driouich et al., 2013). The RET creates a suitable microenvironment for the root, central to its development and function (Nazari et al., 2022).

Since the first description of mucilage-producing cells (later referred to as AC-DCs) (Meyen 1837), the understanding of the composition and role of their secretions has evolved significantly and these are now better characterized (Rovira, 1969; Goldberg et al., 1989; Hawes and Smith, 1989; Hawes et al., 1998; Wen et al., 2007, 2009; Ropitiaux et al., 2019, 2020; Driouich et al., 2021; Fortier et al., 2023; Fourneau et al., 2024). Indeed, root secretions play a major role in the molecular dialogue and the interactions between the plant and beneficial and phytopathogenic microorganisms of the rhizosphere. Root exudates diffuse into the soil, define the rhizosphere and play a significant role in shaping rhizospheric microbiota, and its rhizocompetence (*i.e.*, chemotaxis and growth) thus contributing to root protection (Wen et al., 2009; Cannesan et al., 2011;

Abbreviations: AC-DCs, root associated, cap-derived cells; AGPs, arabinogalactan-proteins; exDNA, extracellular DNA; PGPR, plant growth-promoting rhizobacteria; RET, root extracellular trap.

* Corresponding author.

E-mail addresses: alexia.gaudry2@univ-rouen.fr, gaudry.alexia@hotmail.fr (A. Gaudry).

<https://doi.org/10.1016/j.rhisph.2025.101016>

Received 22 October 2024; Received in revised form 18 December 2024; Accepted 2 January 2025

Available online 5 January 2025

2452-2198/© 2025 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Haichar et al., 2014; Bani et al., 2018; Tian et al., 2020; Ma et al., 2022; Fournneau et al., 2024). Initially, the mucilage of the RET, was thought to function as a lubricant, protecting roots during its penetration and growth within the soil. However, a number of recent studies have clearly shown that the RET ensures much more complex functional roles in relation with plant health and survival. RET mucilage components, such as extracellular DNA (ex DNA) and arabinogalactan-proteins (AGPs) were shown to attract, repel or limit the growth of several microorganisms including fungi, bacteria and oomycetes (Cannesan et al., 2012; Tran et al., 2016). Other studies have also shown that the RET can act as a physical barrier, or a decoy that traps and promotes cell lysis of pathogenic microorganisms, thereby protecting the root from infection (Goldberg et al., 1989; Wen et al., 2009; Cannesan et al., 2012; Tran et al., 2016; Ropitiaux et al., 2020).

The microbial community of the rhizosphere contains a diversity of microorganisms including beneficials such as Plant Growth-Promoting Rhizobacteria (PGPR), neutral microorganisms and pathogens such as oomycetes, whose balance is important for plant health (Ali et al., 2017; Mendes et al., 2013). For the colonization of the rhizosphere and roots, motility and chemotaxis give to the motile microorganisms a substantial advantage whether they are beneficial or pathogenic to the plant (Feng et al., 2021; Kasteel et al., 2023). Chemotaxis allows motile microorganisms to explore the environment, sense and respond to a chemical gradient by migrating towards attractive molecules or moving away from repellent ones (Bi and Sourjik, 2018). By adjusting the frequency of their runs and tumbles, microorganisms could migrate from the bulk soil to the rhizosphere under the influence of root secretions.

In this work, we investigated the role of the RET of pea (*Pisum sativum*) and soybean (*Glycine max*) in the interactions with two flagellated PGPR: *Bacillus subtilis* and *Pseudomonas fluorescens*, and with the zoospores of the oomycete *Phytophthora parasitica* (Meng et al., 2014; Yadav et al., 2021; Yu et al., 2021). We studied the effect of the RET on the access of these microorganisms to the root tip and their behavior by determining their distribution, swimming speeds and trajectories, either within or outside the RET. Specifically, we aimed to test different types of microorganisms with distinct characteristic, such as steric hindrance and taxonomic classification. *B. subtilis* and *P. fluorescens* are bacteria that differ in Gram classification (Gram-positive and Gram-negative bacteria respectively), while *P. parasitica* is an eukaryotic species (an oomycete). The three microorganisms also vary significantly in cell body size, as well as in molecular regulation, structure, insertion and number of flagella (Hua et al., 2008; Piggot, 2009; Dodd, 2014; Aponte et al., 2017; Bassani et al. 2020a, 2020b; Liu et al., 2022; Tran et al., 2022; Kasteel et al., 2023). Our findings show that the RET strongly influences the access and the movement of the three microorganisms, although not in the same way. We show that inside the RET, the speed of *B. subtilis* is reduced by three-folds and that of *P. parasitica* zoospores by nine-fold, and their trajectories are strongly altered as compared to those of microorganisms that are not in contact with the root tip.

2. Materials and methods

2.1. Plant material

Pea seeds (*Pisum sativum*, cv. Astronaute) (RAGT, Rodez, France) were sterilized for 10 min with 70% aqueous ethanol for 10 min and with sodium hypochlorite (1% commercial bleach, 9.5% active ingredient) for 10 min. Soybean seeds (*Glycine max*, cv. Nessie PZO) (Sempartners, Maule, France) were sterilized twice overnight with chlorine gas. Sterilized pea and soybean seeds were rinsed five times with sterile water then soaked in sterile water at room temperature overnight in the dark. Seeds were sown on square Petri dishes containing 40 mL of solid half strength Murashige and Skoog (MS) agar 1% medium (Duchefa Biochemie). These dishes, containing two lines of six seeds for pea and two lines of five seeds for soybean, were placed upright at 21 °C for pea for 4 days and at 24 °C for soybean for 3 days (cycle of 16 h day and 8 h

night).

2.2. Microbial culture conditions

Pseudomonas fluorescens strain ATCC 17400 was provided by Pr. Pierre Cornelis (Université Libre de Bruxelles, Belgium) and *Bacillus subtilis* strain ATCC 6633 was purchased from the American Type Culture Collection (ATCC). Bacteria were grown in Luria-Bertani (LB) medium (Lennox, Duchefa Biochemie) at 30 °C under agitation at 130 rpm. When a high density of bacterial suspension was used ($N > 10^9$ CFU/mL), bacteria were grown overnight in LB medium at 30 °C. When a low density of bacterial suspension was used, bacteria were grown from an overnight culture in LB medium at 30 °C to $10^6 < N < 10^7$ CFU/mL, then the suspension was diluted 10-fold in phosphate buffer saline (PBS 1x, pH 7). To determine cell densities, we cultured bacteria and correlated the optical density at 580 nm (OD_{580nm}) with bacterial density (N in CFU/mL) by establishing the proportionality factor specific to the spectrophotometer used in the experiments. Bacterial density was then monitored by measuring the OD_{580nm} .

Phytophthora parasitica (isolate INRA-310) was provided by Dr. Agnès Attard (Sophia Antipolis, INRAe, France). The oomycete was grown on V8 agar medium at 25 °C in the dark and to produce zoospores, the mycelia were grown in V8 liquid medium at 25 °C under continuous light for a week. Mycelia were harvested from the rich medium and transferred on water supplemented with 2% agar for 4 days (Attard et al., 2010; Galiana et al., 2005). Zoospores were released as described by Galiana et al., (2005). Petri dishes containing mycelia were placed at 4 °C for 30 min, then 10 mL of distilled water was added and incubated for another 30 min at room temperature in the dark. For confrontation tests, 10^5 zoospores/mL of water, counted using a Malassez counting cell, were added to root tips.

2.3. Confrontation tests between soybean and pea root tips and microorganisms

For observations, root tips of pea and soybean were mounted on a 10-well Teflon microscope slide and, after rinsing with a PBS 1x (pH 7), 20 μ L *P. fluorescens* suspension (an overnight culture) or *B. subtilis* (an overnight culture or culture to $10^6 < N < 10^7$ CFU/mL diluted 10-fold in PBS) or 20 μ L of water containing *P. parasitica* zoospores (about 10^5 zoospores/mL of water) were added to each well. To compare their behavior, we selected areas distant from the RET, as well as areas with AC-DCs as defined for both the outside and inside of the RET respectively. For microorganisms alone, 5 μ L of microbial suspension were added to a 10-well Teflon microscope slide. To test an inert surface as a control, 1 μ L glass microcapillary (Microcapillary Tube, Drummond Microcaps®) were cut to a length of 5–7 mm and sealed with a flame. Before use, the microcapillaries were degreased in successive baths of acetic acid (2%) and ethanol (70% and 90%). Observations were made under a bright-field microscope (Leica DMI6000B, Leica Microsystems, Wetzlar Germany or Zeiss CellDiscoverer 7, Carl Zeiss AG, Oberkochen, Germany) for 5–30 min after inoculation.

2.4. Tracking of microorganisms and data treatment

Videos of 10 s of each region of interest were recorded using a Leica DMI6000B or a Zeiss Cell Discoverer 7. The frame rate was about 17 frames/s and the size of view fields was from 1280 x 960 pixels (videos) and 3840 x 2820 pixels (images) for the Leica DMI6000B and 1196 x 1196 pixels (videos and images) for the Zeiss Cell Discoverer 7. Tracks were processed with Fiji using the MTrackJ plugin. For trajectories of at least 1 s, the speed was extracted from the software for each track. The travelled distance was the “length of the trajectories” travelled by the microorganisms (cumulative distances), corresponding to the total distance travelled between the start point (first point) and the end point after 4 s for bacteria and 2 s for zoospores. A random sampling of 50

tracks was used for the calculations. Directness was calculated as $D = d_{\text{euclidian}}/d_{\text{cumulative}}$ on 50 tracks of at least 1 s from each condition. $d_{\text{euclidian}}$ is the euclidian distance between the first and the last points. $d_{\text{cumulative}}$ is the total distance covered by the microorganism between the start point and the end points (Cohen et al., 2023). Boxplots were generated using Rstudio software (<http://www.rstudio.com/>) and histograms were generated using Excel software (<https://office.microsoft.com/excel>).

2.5. Statistical analysis

Statistical significance was calculated using the Kruskal–Wallis test with Dunn's *post hoc* multiple comparison test, applying the Bonferroni correction, and $P \leq 0.05$ was accepted as significant. Statistical analyses were performed using Rstudio software (<http://www.rstudio.com/>).

3. Results

3.1. The RET modifies the distribution of microorganisms around the root tip

To facilitate microscopical observations of the distribution of bacteria around the root tip, we used a dense bacterial suspension (an overnight culture). For *Phytophthora parasitica* zoospores, we used a suspension of 10^5 zoospores/mL. To perform the confrontation test between the root and the microorganisms, the microbial suspension was added to the root prior to microscopical observations.

The confrontation test with *Pseudomonas fluorescens* showed that the penetration of bacteria into the RET was clearly hindered (Fig. 1a-c). Significant changes in the density and velocity of the bacteria were visible under the microscope (Fig. 1a-c, Vid. 1, Vid. 2). Bacteria were filtered through the RET and had a heterogeneous accumulation around it. They also formed aggregates at the periphery of the network (Fig. 1b, Vid. 1). Bacteria present inside the RET were visibly slower and much less motile than bacteria found outside the RET (Vid. 1, Vid. 2). The behavior of the bacteria outside the RET was similar to that of bacteria alone. The bacteria were highly mobile and separated from each other, not forming aggregates (Fig. 1c, Vid. 2). These bacterial behaviors were similar for both plant species, pea (*Pisum sativum*) and soybean (*Glycine max*).

Supplementary video related to this article can be found at <https://doi.org/10.1016/j.priml.2019.12.005>

The confrontation test with *Bacillus subtilis* revealed that the bacteria were distributed in three distinct areas around the root tip (Fig. 1d-g): *i*) a first zone far from the root tip where the bacteria exhibited high mobility and high density with sporadic bacterial accumulation (Fig. 1d-f, a dark area, Vid. 3), *ii*) a second zone presenting a small number of bacteria (very low density) with no bacterial aggregation (Fig. 1d-f, a clear area, Vid. 3). This area was almost bacteria free, and *iii*) a third zone within the RET, through which the bacteria either moved very slowly or were immobile forming a large number of aggregates (Fig. 1d-g, Vid. 3). As for *P. fluorescens*, the distribution pattern of *B. subtilis* was similar in both plant species, pea and soybean.

Supplementary video related to this article can be found at <https://doi.org/10.1016/j.priml.2019.12.005>.

Thus, the two bacteria exhibited different behaviors in the presence of the root. The movement of *B. subtilis* was altered at a greater distance from the root tip compared to *P. fluorescens*, likely due to the effect of molecules diffusing from root tip cells, at a point where the presence of the RET is unlikely. Although both bacterial species formed aggregates, these occurred at different locations within and around the RET.

The confrontation between the root tip and *P. parasitica* zoospores revealed that these were distributed at a constant density around the root tip, despite variations in their behavior (Fig. 2a). Zoospores exhibited moderate mobility when located away from the root tip (Vid. 4). Some zoospores accumulated in certain areas quite far from the root

tip, mostly at the periphery of the observation zone, near the edge of the microscope slide well (Fig. 2b). Closer to the RET, the zoospores were very slow, exhibiting a similar density and did not delimit the RET zone (Fig. 2a, Vid. 4). Once within the RET (in direct contact with the root tip or the AC-DCs), most of the zoospores encysted in less than 5 min after inoculation before germinating (Fig. 2c and d), and some of them underwent cell lysis and death (Fig. 2c).

Supplementary video related to this article can be found at <https://doi.org/10.1016/j.priml.2019.12.005>.

3.2. The RET influences microbial trajectories

To further study the movement of the microorganisms around the root tip, we captured videos and tracked *B. subtilis* and *P. parasitica* zoospores using the MtrackJ plugin from Fiji. In these experiments we were not able to track *P. fluorescens* due to its small size. We attempted to stain the bacteria with fluorescent dyes to make them more visible (data not shown), but the stain was not effective enough to clearly visualize *P. fluorescens* and *B. subtilis* motility was altered significantly.

To characterize and quantify the motility of the microorganisms, new confrontation tests were performed on *B. subtilis* with a less dense suspension ($10^5 < N < 10^6$ CFU/mL) and a suspension of *P. parasitica* zoospores (10^5 zoospores/mL). We had preliminarily checked that the behavior of the bacteria did not change according to suspension density and/or culture growth phase.

Vid. 5 shows the typical trajectories observed for the two microorganisms in the presence of the root tip (outside and inside the RET) or in control conditions (in contact with a microcapillary or alone). *B. subtilis* displayed straight-line trajectories when observed alone (Vid. 5a), in contact with a microcapillary (Vid. 5c) or outside the RET (Vid. 5e). In contrast, the bacteria had very compacted trajectories inside the RET (Vid. 5g). In the case of *P. parasitica*, the zoospores displayed quite straight-line trajectories when they were alone (Vid. 5b) or in contact with a microcapillary (Vid. 5d). On the other hand, outside the RET (Vid. 5f) and inside the RET (Vid. 5h), the zoospores make numerous changes in direction or exhibit constricted trajectories. Clearly, the microorganisms seem to exhibit similar behaviors on both pea and soybean plants. Quantification of these observations were made and the data are presented below.

Supplementary video related to this article can be found at <https://doi.org/10.1016/j.priml.2019.12.005>.

3.3. The RET and its surrounding environment reduce the speed of the microorganisms

We determined the velocity of both *B. subtilis* bacteria and *P. parasitica* zoospores around the root tip for both plant species (Fig. 3a and b). As shown in Fig. 3a, the average velocity of *B. subtilis* alone (19 $\mu\text{m/s}$) was not statistically different from that of bacteria in contact with a microcapillary (17 $\mu\text{m/s}$) or outside the RET (*i.e.*, away from the root tip) of soybean or pea (20 $\mu\text{m/s}$ each). In contrast, inside the RET (*i.e.*, close to the AC-DCs) of both plant species, bacteria displayed a velocity of 6 $\mu\text{m/s}$ which is three times slower than the bacteria alone or present outside the RET.

P. parasitica zoospores swim faster than for *B. subtilis* (Fig. 3b). The zoospores alone had the highest velocity at 217 $\mu\text{m/s}$, whereas those in contact with a microcapillary had a velocity of 201 $\mu\text{m/s}$. In contrast with root tip, the zoospores' speed dropped significantly. Outside the RET, zoospores were slower than zoospores alone (103 $\mu\text{m/s}$ and 102 $\mu\text{m/s}$ for soybean and pea, respectively), but still four times faster than those inside the RET (18 $\mu\text{m/s}$ and 32 $\mu\text{m/s}$ for soybean and pea, respectively). Thus, like bacteria, *P. parasitica* zoospores had the lowest velocity inside the RET.

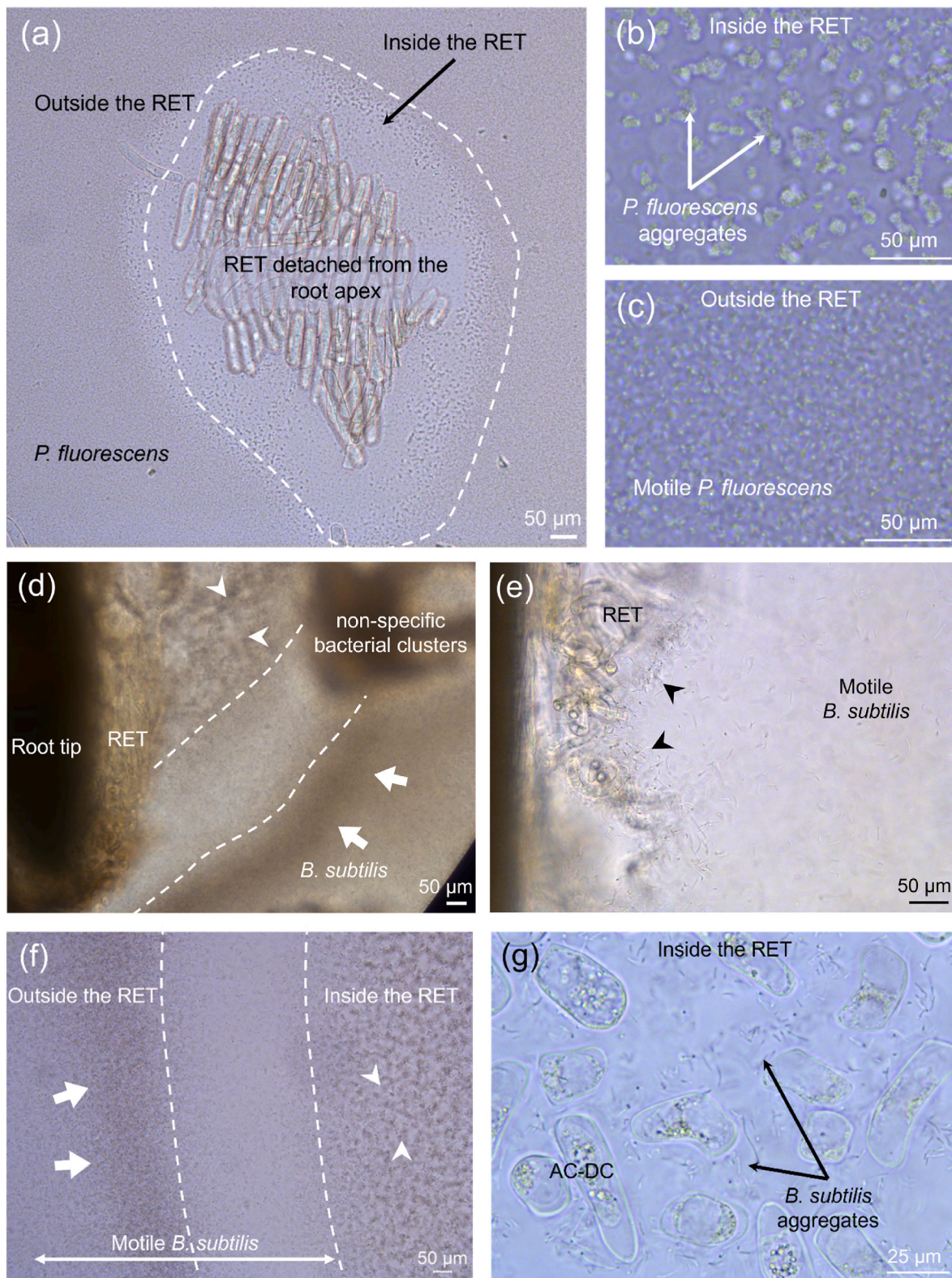


Fig. 1. Distribution of bacteria around the root tip and the RET. (a–c) Distribution of *Pseudomonas fluorescens*. The distribution of the bacteria occurs in two zones (dashed lines) visible in (a). (d–g) Distribution of *Bacillus subtilis*. The distribution of the bacteria occurs in three zones (dashed lines) visible in (d) and (f). Arrows indicate accumulation of bacteria. Arrowheads indicate bacterial aggregates. The presence of non-specific bacterial clusters (d) is caused by the high bacterial density. Scale bars = 50 μm (a–f), 25 μm (g). AC-DCs: root associated, cap-derived cells; RET: root extracellular trap.

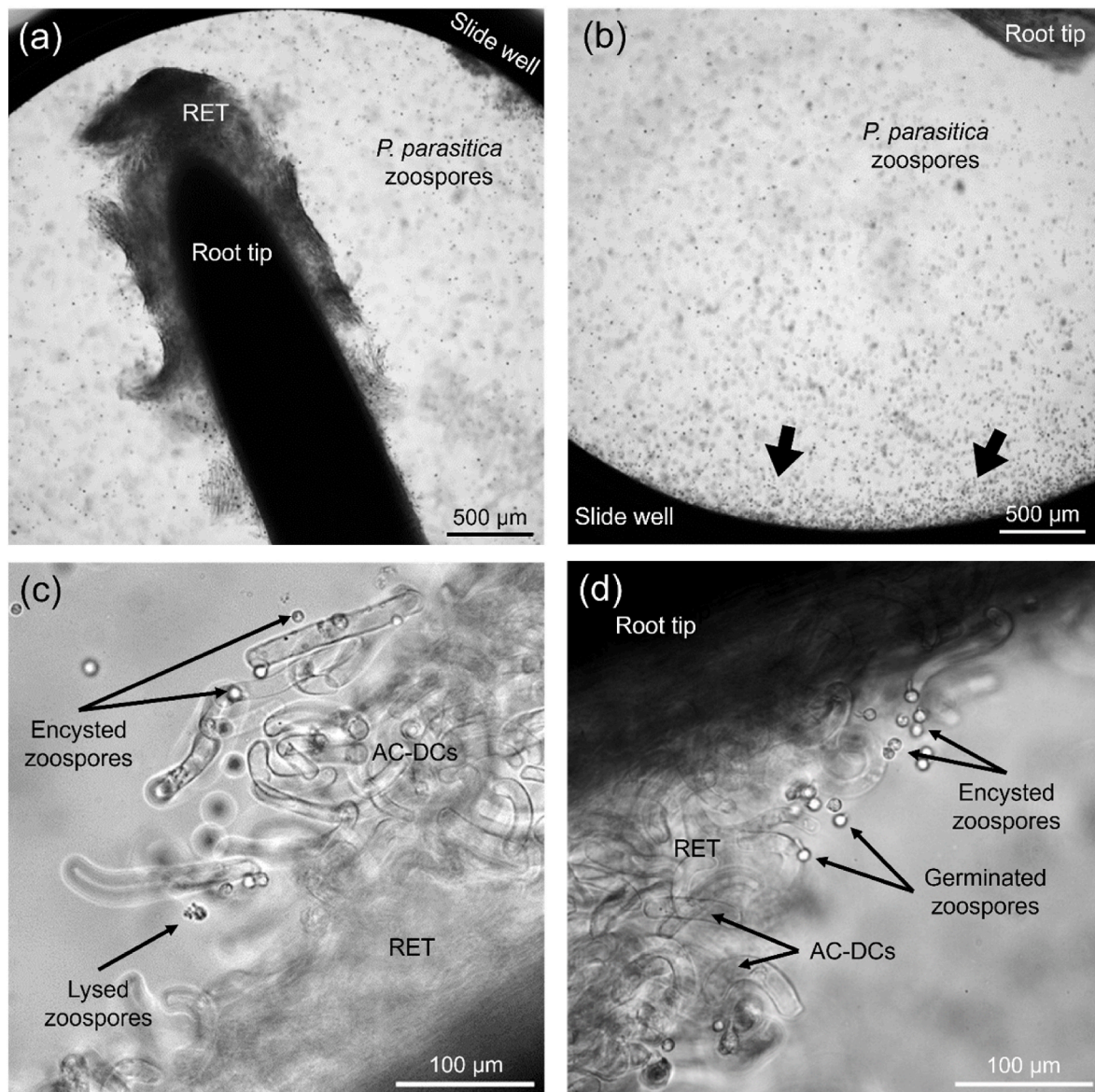


Fig. 2. Distribution of *Phytophthora parasitica* zoospores around the root tip and the RET. Observations show that zoospores are homogeneously distributed around the root tip (a), but can accumulate further along at the well edge (arrows) (b). Encysted and germinated zoospores are seen in contact with the root tip or AC-DCs (c–d). Scale bars = 500 μm (a–b), 100 μm (c–d). AC-DCs: root associated, cap-derived cells; RET: root extracellular trap.

3.4. The RET alters the distance travelled by the microorganisms and their directness

The data showing the effect of RET on the directness and trajectory lengths of the microorganisms are presented in Fig. 3c–f. We found that *B. subtilis* alone were able to cover a distance of 77 μm in 4 s, which was statistically identical to that covered by bacteria in contact with a microcapillary (73.5 μm) or present outside the RET of soybean or pea (90 μm and 74.5 μm for soybean and pea, respectively) (Fig. 3c). Bacteria inside the RET had the shorter distance travelled (18 and 12.5 μm for soybean and pea, respectively) (Fig. 3c). These observations were concomitant with the directness of the bacterial trajectories (Fig. 3e). The directness (D) was calculated and classified into two categories as described by Cohen et al. (2023), namely straight-line trajectories ($D > 0.8$) and curved trajectories ($0 < D < 0.5$). When *B. subtilis* were alone (72%), in contact with a microcapillary (40%) or outside the RET of both plant species (64% for soybean and 60% for pea) most of their trajectories were straight-line. Bacteria located inside the RET moved mostly with a weak directness typical of curved trajectories (86% and 84%,

respectively for soybean and pea).

Fig. 3d shows that the zoospores alone and those in contact with a microcapillary travelled a similar distance in 2 s (467 μm and 426 μm , respectively). Outside the RET of soybean and pea, the zoospores covered a distance twice as short as the zoospores alone (222 μm and 249 μm , respectively), but they travelled a distance five times as long as the zoospores inside the RET of soybean and pea (46 μm and 53 μm , respectively) (Fig. 3d). Regarding the directness of zoospore trajectories, when the zoospores were alone, they displayed mostly straight-line trajectories (58%) (Fig. 3f). The zoospores in contact with a microcapillary showed approximately equal proportions of low and high directness, each around 40%. Unlike *B. subtilis*, the zoospores outside and inside the RET of soybean and pea had largely a weak directness (from 74% to 98%) and exhibited a curved trajectory pattern (Fig. 3f).

Together these results indicate that the RET caused a reduction in speed and affected the trajectories of the microorganisms. These changes resulted in more restricted movements, with shorter distances travelled and less linear trajectories once the microorganisms had successfully entered the RET. However, the first impact on microbial behavior

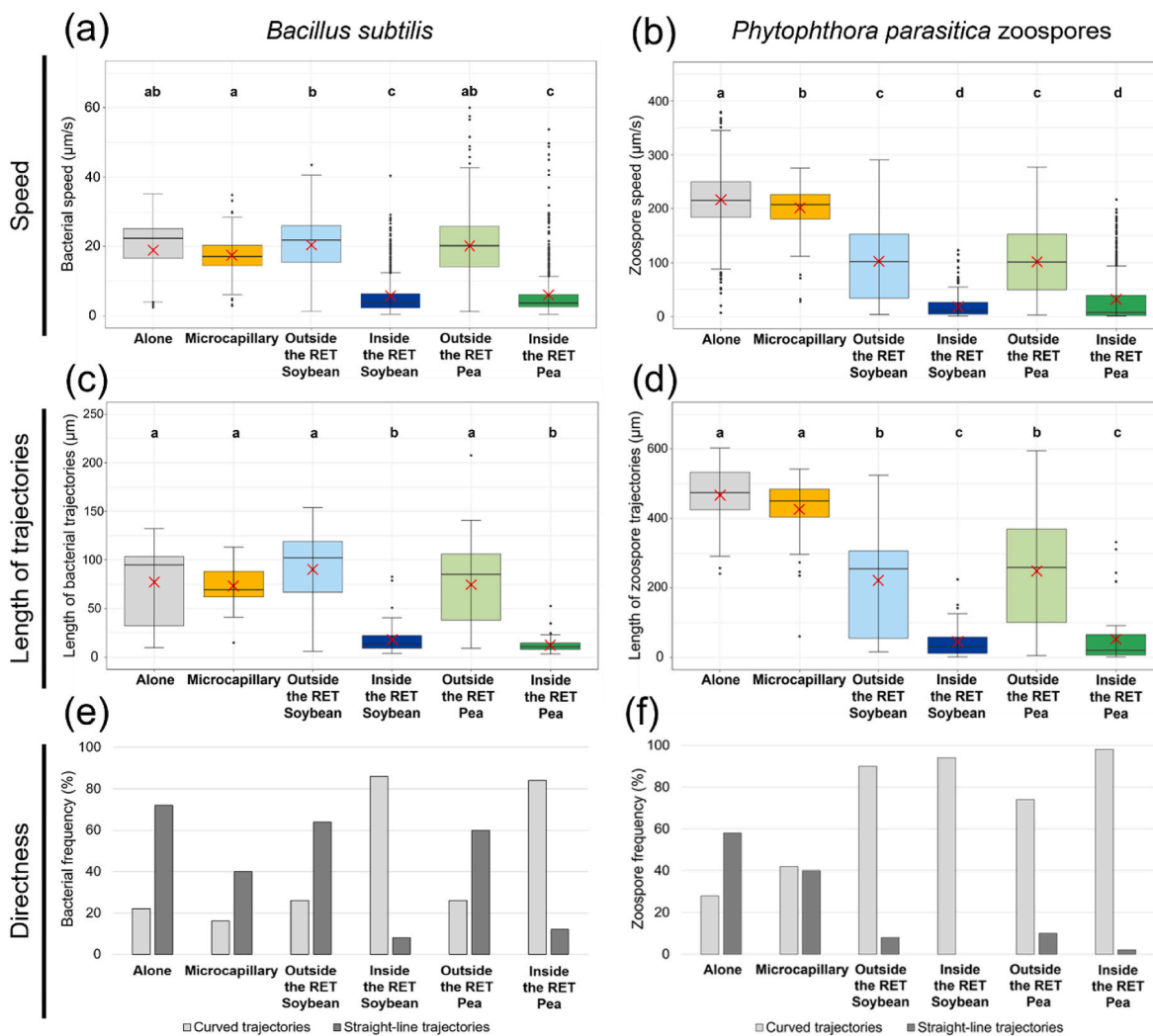


Fig. 3. Kinetics parameters measured for *Bacillus subtilis* and *Phytophthora parasitica* zoospores. (a, c, e) Parameters measured for *Bacillus subtilis*. (b, d, f) Parameters measured for *Phytophthora parasitica* zoospores. (a–b) Velocity measured according to each condition. $305 < n < 2399$. (c–d) Length of trajectories determined over 4 s for bacteria and 2 s for zoospores. $n = 50$ for each condition. (e–f) Directness of microbial trajectories. Curved trajectories = $0 < D < 0.5$ and straight-line trajectories = $D > 0.8$. $n = 50$ for each condition. Letters indicate the result of the statistical Dunn's multiple comparison Test with $P \leq 0.05$. Red crosses indicate the mean speed. No statistical differences were found between for the two Fabaceae. RET: root extracellular trap.

(change of density or movement) occurred quite away from the root tip and the RET. Therefore, the behavioral changes observed may not be due to the sole effect of RET. Hence, the release of molecules by root cells (*i.e.*, root secretions) is likely to contribute to the observed effects on the migration and access of microorganisms to the root tip.

4. Discussion

4.1. The RET does not act fully as a protective filter against microorganisms

Our observations show that the RET of soybean and pea affects the behavior and access of the three microorganisms used in this study to the root tip, but does not totally prevent them from passing through. The RET, a structure known to protect the root tip (Druiouich et al., 2021), would therefore not act as an impenetrable barrier, but rather as a physical and/or chemical filter that would modulate the access of pathogenic or beneficial microorganisms to the root. To maintain a healthy status, plants must sustain a balanced rhizosphere microbiota (Hassan et al., 2019). At a local level, the RET likely contributes to this balance by restricting the migration of microorganisms toward the root tip through the production of a complex matrix that limits their

movement. Being a vulnerable zone essential to development of the root and, consequently the entire plant, the root tip requires an efficient protection. Similarly to the gut microbiota, achieving a balance among different microbial communities, including beneficial ones, is crucial to ensure the organism's health and prevent dysbiosis (Das and Nair, 2019; Fassarella et al., 2021). However, in addition to RET and its components, other root secretions would also contribute to the observed effects on the behavior of the microorganisms during their interaction with the root tip. A model summarizing these effects is presented in Fig. 4.

The two bacteria do not have the same distribution profiles around the RET. *Pseudomonas fluorescens* accumulates around the RET, creating a counter-coloration that appears to delineate the network. On the surface of the RET, some bacteria form aggregates, while others remain mobile and seem unaffected. Among the latter, some managed to slip inside the network and had a reduced speed (Fig. 4). Unfortunately, measurements of speed and trajectory could not be carried out on this bacterium because of its small size (rod-shaped, about 1–3 µm long) (Aponte et al., 2017; Ito et al., 2007). Nevertheless, even in the absence of quantitative data, it is possible to suggest that RET has a filtering effect on this microorganism, reducing the number of bacteria reaching the root tip, although it is a beneficial bacterium.

Bacillus subtilis, (rod-shaped, about 2–6 µm long) (Liu et al., 2022)

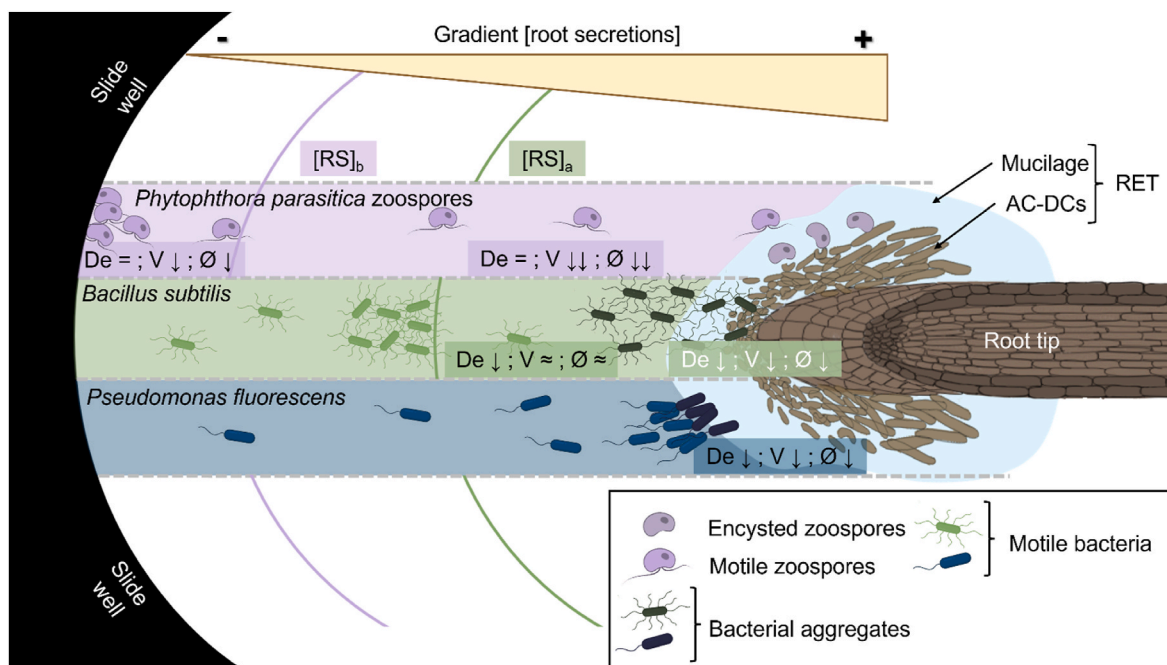


Fig. 4. Schematic representation showing the effect of root secretions on the behavior of three microorganisms and their access to the root tip. RS: root secretion, [RS]_a and [RS]_b represent two distinct concentrations of the proposed gradient at which the behavior of *Bacillus subtilis* or *Phytophthora parasitica* zoospores changes, with [RS]_a > [RS]_b, De: microbial density, V: observed or calculated velocity, Ø: measured length and directness of the trajectories, = : no change, ≈: little change, ↓: moderate decrease, ↓↓: strong decrease. Variations are shown in comparison with microbial suspensions (microorganisms alone, in the absence of root tip).

also undergoes a filtering effect with a reduction in the number of bacteria reaching the root tip in three distinct steps (Fig. 4). Indeed, an initial change in *B. subtilis* behavior occurs quite far from the root tip (100 µm to > 500 µm), leading locally to a marked increase in bacterial density, but without visibly affecting bacterial speed. Surprisingly, this accumulation of bacteria occurs quite far from the RET and therefore cannot be explained by direct interactions with the network. The downstream zone is much less dense, confirming that they are hindered in their progress towards the RET. In contact with the root tip and AC-DCs, the motility of *B. subtilis* decreases considerably, or even come to a complete standstill. Also, the bacteria exhibit short and compacted trajectories or form aggregates.

Phytophthora parasitica zoospores have a kidney-shaped cell body of about 10 µm in diameter and are more sterically hindered compared to bacteria (Mitchell et al., 2002; Tran et al., 2022). Despite this, the zoospores manage to penetrate the RET of soybean and pea, where some encyst (Fig. 4). The speed of zoospores closest to the root tip decreases by a factor of four compared with the speeds observed at the periphery of the well, and by a factor of nine compared with the zoospores alone. The length and shape of the trajectories confirm these observations, since they are shorter and more curved in the presence of the root, especially inside the RET. Unlike *B. subtilis*, the presence of the root tip affects the movement of all zoospores, even those farthest away, reducing their speed to half that of the zoospores alone.

The present work characterized for the first time the effect of the RET, on the speeds and trajectories of *B. subtilis* and *P. parasitica* zoospores. Gochnauer et al. (1990) and Shirakawa et al. (2023) have shown a difference in motility when microorganisms are inside the RET, but to our knowledge no study has quantified the impact of the network on microbial movement.

How can these differences in behavior be explained? The simplest hypothesis would be a steric exclusion phenomenon due to the mesh size of the reticulated network formed by the RET polymers (Tran et al., 2016; Ropitiaux et al., 2020; Shirakawa et al., 2023). But, the RET filtering effect observed for the two bacteria cannot be due to this simple mechanism, as the zoospores of *P. parasitica*, which are bulkier than the

bacteria, do not undergo this effect. In addition, the first impact of the presence of the root tip on the behavior of *B. subtilis* (bacterial accumulation followed by a drop in bacterial density) and *P. parasitica* (drop in movement speed and change in trajectories) appear in areas relatively far from the root tip and, probably, from the RET. On the other hand, the RET is most likely not homogeneous, that there are several levels of cross-linking and that “tunnels and walls” run through it, forming a sort of labyrinth. These super-structural “tunnels” could form wide, favorable “roads” for the circulation of microorganisms inside the RET. However, the walls of these “tunnels”, particularly those woven from polysaccharide fibers, are favorable surfaces for interactions with circulating microorganisms. Tan et al. (2016) observed that certain wall components such as pectins and xyloglucan, have an impact on the attachment of bacteria such as *Salmonella enterica* and *Listeria monocytogenes* to plant cell walls. They showed, for example, that the presence of pectins promotes the attachment of *S. enterica* to these surfaces, but reduces that of *L. monocytogenes*. The authors support the idea that this phenomenon is not solely linked to the physicochemical properties of the bacterial surface or the hydrophobicity of the attachment surface, but rather to the influence of polysaccharides on the physical and structural properties of the latter. These interactions could be chemical, electrostatic, hydrophobic and/or van der Waals (Berne et al., 2018). In addition, RET density may vary with distance from the root tip as found in seed mucilage and could allow microorganisms to penetrate the network (Macquet et al., 2007; Voiniciuc et al., 2015).

4.2. Root secretions are important for root-microorganism interactions

Root exudates, largely involved in root-microorganism interactions, permeate the RET and diffuse beyond it, generating specific or non-specific microbial responses (Sugiyama, 2019; Tian et al., 2020; Feng et al., 2021). We therefore propose to explain our data by the combined action of *i*) the complex network formed by the RET and *ii*) a gradient of root secretions (*i.e.*, root exudates and polymers, alone or in combination, derived from the RET) (Fig. 4).

In the proposed model, *P. parasitica* zoospores appear to be very

sensitive to the gradient of root secretions as their movement is strongly affected. A particular concentration of root secretions (named [RS]_b) would further increase this impact on speed and trajectories. The zoospores then seem to reach and cross the RET without being slowed down again by obstacles or interactions with the polysaccharides in the “tunnels”, for instance. On the other hand, within the RET, they would perceive chemical or mechanical signals that would cause them to encyst rapidly. The density of zoospores remains constant in all zones, but their premature encystment when crossing the RET would slow down their pathogenic action and thus act as a decoy when the germination occurs at the surface of the AC-DCs (Goldberg et al., 1989).

B. subtilis modifies its behavior quite far from the root, at a concentration of root secretions that we have named [RS]_a in our model and which would be higher than that mentioned above for the zoospores (Fig. 4). This bacterium accumulates at that site and, beyond it, the filtering effect begins. However, as mentioned above, it is difficult to assume that the mucilaginous network of the RET extends to that zone. It is therefore conceivable that *B. subtilis* bacteria would encounter one or more molecules in the gradient of root secretions in a sufficient quantity to halt the progress of the majority of them, while a minority would continue to move towards the root at the same speed. Near the root, however, the speed of the bacteria drops and their trajectories are very short. It is thus tempting to explain these observations by the numerous interactions with organic molecules shaping the pathways within the RET and a high concentration of nutrients provided by root exudates. The aggregates observed on the surface of the root tip or AC-DCs possibly correspond to the onset of biofilm formation.

The behavior of *P. fluorescens* is certainly the easiest to integrate into the model. The bacteria move unhindered up the gradient of root secretions, and then accumulate at a distance from the root tip which would therefore correspond to the surface of the RET. Numerous interactions would therefore take place with this surface, leading to sporadic accumulations of bacteria at this level, and even to the formation of aggregates. A minority of bacteria penetrate the network and display a reduced speed as a result of potential interactions with the RET and/or the root exudates.

Furthermore, the phenomena of filtration, aggregation and encystment could be due to a different chemotactic responses of the different microorganisms. Therefore, it is tempting to postulate that there is an important effect of root secreted molecules that diffuse beyond the RET, on the behavior of *B. subtilis* and *P. parasitica* zoospores (Fig. 4). Fang et al. (2016) have shown that certain molecules present in rapeseed exudates, such as benzothiazole and 4-methoxyindole, negatively affect the motility of *P. parasitica* zoospores. However, it cannot be ruled out that other mechanisms may influence the motility of microorganisms, such as the viscoelastic properties of the external environment, as shown by Schwanbeck et al. (2021) on *Clostridioides difficile* and Chaban et al. (2018) on *Wolinella succinogenes*, *Arcobacter butzleri*, *Salmonella enterica* and *Campylobacter jejuni*. In addition, microorganism-specific communication mechanisms, as well as factors such as pH, oxygen level, ions could also play a role as described by Kasteel et al. (2023); Wong-Ng et al. (2018); Laganenka et al. (2016) and Galiana et al. (2008). Galiana et al. (2019) and Lupatelli et al. (2023) showed that a particular concentration of a potassium gradient induces *P. parasitica* zoospores aggregation and their encystment.

4.3. The behavior of microorganisms is not plant species-specific

The microbial behaviors we observed are identical in the RET of pea and soybean, although the composition of the mucilage is different (Ropitiaux et al., 2019, 2020). However, it is possible that the structural heterogeneity of the networks and the size of “tunnels and walls” proposed in our model are similar. One would have expected that interactions with diffusible root secretions and/or with the “walls” in the “labyrinth” would be different for each plant, but the molecules or association of molecules responsible for the observed behaviors may be

present in both Fabaceae species. It would be very interesting to apply the methodological strategy developed in this work to other plant models, such as grasses, which have a different RET composition than the Fabaceae species (Nguyen et al., 2024).

Interestingly, some of the microbial behaviors we observed in the present work have also been studied in certain plant species. In their study Hawes and Pueppke (1987), showed a correlation between the aggregation and filtration of *Agrobacterium tumefaciens* bacteria around the AC-DCs of several plant species. In this way, AC-DCs from plants that are not susceptible to the phytopathogenic bacterium form an exclusion zone around them, limiting bacterial access and adhesion to plant cells. A little later, the work of Gochbauer et al. (1990) on maize also illustrated the same filtration phenomenon, under different conditions, on certain bacteria such as *Rhizobium* sp., *Bacillus* sp. and *Escherichia coli*, while other bacteria such as *P. fluorescens*, *Cytophaga* sp. and *Streptomyces* sp. could reach the AC-DCs. The authors did not find any evidence of bacterial aggregates under their conditions. This difference in behavior between the different species could be attributed to their different response and/or level of sensitivity to the chemical gradients present around the root tip, which would influence their movement and distribution.

The behavior of *P. parasitica* zoospores differs slightly from that described by Fang et al. (2016) and is very different from that shown by Ropitiaux et al. (2020). The first authors showed that *P. parasitica* zoospores are attracted to the oilseed rape root, accumulate and encyst at the periphery of the root tip after only 5 min of incubation. Although the authors did not mention the presence or absence of RET, examination of their micrographs suggest that the zoospores could be filtered by the network and the encyst on its surface.

The second authors observed that when in contact with the root tip of soybean (cv. Castetis), *P. parasitica* zoospores were unable to penetrate the network. Outside the RET, the behavior of almost all of them seemed similar to that of the zoospores alone (Ropitiaux et al., 2020). The authors did not observe any accumulation at the edge of the observation zone or any encystment of zoospores inside the network. On the contrary, the zoospores seemed to bounce off the RET, which is what we have observed when zoospores were put into in contact with microcapillaries. However, when the zoospores managed to enter the network, they lysed immediately. In the present study, cell lysis also occurred although in a very limited number of cysts.

In a microfluidic study, Cohen et al. (2023) recently reported that zoospores of *P. parasitica* detected the presence of the root tip of *A. thaliana* at a distance of about 300 μm, with a consequent alteration in their movement (drop in speed and increase in the proportion of curved trajectories). Under the experimental conditions of that study, the RET appeared to be scarce (only a few AC-DCs were observed) and the results suggested that the reduction of the motility of zoospores was induced via the perception of root exudates. On the other hand, the aggregation phenomenon does not seem to be linked to the detection of a signal from the root, but rather to a signal released by the zoospores themselves. Our data on pea and soybean show that *P. parasitica* zoospores can detect root secretions at a much greater distance (more than 3 mm). However, given the plant species the size of the root tip was very different. In addition, RET was very abundant in our conditions. Thus, the quantity and the quality of the root secretions present in the gradient were certainly greater in this case, which would explain this difference in distance of perception by the zoospores. Finally, zoospores may have a different sensitivity to the molecular cocktails released from the roots of different plants.

4.4. RET mucilage may provide a favorable niche for microorganisms

We showed that despite the physical and/or chemical filtration of microorganisms, some of them could penetrate the network. The formation of aggregates on the surface and/or inside the RET suggests that bacteria could begin a process of self-assembly to form biofilms. This

suggests that bacteria could potentially colonize the network and thus serve as a primary inoculum for the rhizosphere microbiota as the root develops in the soil. However, within the RET, certain compounds are known to have an antimicrobial effect and these include Pathogenesis-Related (PR) proteins such as defensins, and ex DNA (Driouich et al., 2021). Wen et al. (2009) and Tran et al. (2016) have shown that ex DNA protects pea seedlings against *Nectria haematococca* and *Ralstonia solanacearum*, thereby limiting root infection. Despite the presence of these compounds with antimicrobial activity, we did not observe any significant cell lysis in either *P. parasitica* or *B. subtilis*, at least during the initial observation period (less than 30 min). In the case of *P. fluorescens*, this is more difficult to confirm by a simple observation because of its small size. It would therefore be necessary to carry out other tests to verify its viability. However, it cannot be ruled out that longer incubation times would have a negative impact on cell viability. In their study, Shirakawa et al. (2023) also observed that the rhizobacteria were trapped and immobilized by RET of *Pinus densiflora* and remained alive even after 48 h of incubation. Therefore, we propose that, in addition to playing a direct role in plant defense by restricting the access of microorganisms to the root tip, the RET may also act as an ecological niche favorable to a controlled installation of microorganisms. These microorganisms could potentially contribute to the root tip defense, most likely via the action of PGPR. Several studies provide support to this hypothesis. For example, Knee et al. (2001) showed that *Rhizobium leguminosarum*, *Pseudomonas fluorescens* and *Burkholderia cepacia* were able to grow using pea mucilage efficiently as their sole carbon source. Beauregard et al. (2013) demonstrated that wall glycomolecules such as pectins, AGPs and xylans, which are also found in the RET, induce biofilm formation in *B. subtilis*. The authors also showed that the bacterium can hydrolyze these molecules and use the sugars as a carbon source, but also as a source for the production of exopolysaccharides, which are involved in biofilm formation. Moreover, in their work, Fournau et al. (2024) demonstrated that *P. fluorescens*, *B. subtilis* and *Azospirillum brasilense* could grow and produce biomass using only root exudates. Studies have also highlighted the novel presence of diazotrophic bacteria in the aerial mucilage of maize and *Heterotis rotundifolia* (Deynze et al., 2018; Amicucci et al., 2019; Pang et al., 2023). This mucilage provides a favorable microhabitat for these bacteria, which in exchange allow atmospheric nitrogen fixation. Finally, in their study, Nazari et al. (2022) showed that root mucilage shares physical and chemical characteristics similar to the exopolysaccharides present in bacterial biofilms, particularly in terms of viscosity and surface tension, thus providing an environment similar to that of biofilms.

4.5. Root mucilage and intestinal mucus layer, a functional convergence?

Like the rhizosphere, the gut lumen contains many bacterial species that form the gut microbiota (Mendes et al., 2013; Schroeder, 2019). Parallels can be made between gut mucus and root mucilage, both in composition and in their putative functions. Intestinal mucus is a defensive barrier preventing the translocation of commensal and pathogenic microorganisms across the mucosal barrier, thereby protecting the intestinal epithelium (Schroeder, 2019). Composed of glycans and mainly mucin, the mucus, like RET mucilage, forms a protective layer that covers the intestine, offering protection to the epithelial cells against dehydration, digestion-related damage and pathogens (Sicard et al., 2017; Driouich et al., 2021). For example, in the colon, mucus is mainly composed of MUC2 mucins, produced by goblet cells and, like seed mucilage, is composed of two distinct layers with different properties. The outer mucus, the less dense layer, allows bacteria to pass through, while the inner layer, firmly attached to the calciform cells, is denser than the outer layer and devoid of bacteria. This mucus forms a physical barrier, that blocks the access of both beneficial and pathogenic bacteria to the epithelium cells (Johansson et al., 2011; Johansson and Hansson, 2016; Valibeknejad et al., 2023). Other studies also show that the gut mucus significantly slows down flagellated bacteria and then

traps them. Indeed, this protective barrier leads to a significant reduction in the efficiency of infection of mice by the pathogenic bacterium *Salmonella typhimurium*, via trapping and immobilizing bacteria (Furter et al., 2019).

These observations are in line with what we have observed in our present study. Additionally, unlike the mucus in small intestine, which contains antimicrobial peptides that eliminate microorganisms upon contact with epithelium, colon mucus produces peptides that bind and aggregate bacteria. This aggregation inhibits bacterial extrusion by blocking them due to their physical size, restricting their mobility, and acting as both a chemical and physical filter. Interestingly, small peptides (e.g., defensins) are also found in root mucilage and might perform a similar role to those in colon mucus, leading to microbial aggregation (Okumura et al., 2016; Sicard et al., 2017; Schroeder, 2019).

At the same time, intestinal mucus, like root mucilage, appears to provide a favorable ecological niche for microorganisms. Mucins are glycoproteins composed mainly of O-glycans (about 80% of their molar mass) containing different sugars including galactose, N-acetylgalactosamine, N-acetylglucosamine and sialic acid (Schroeder, 2019). Thanks to their hydrolytic enzymes, both pathogenic and commensal bacteria are able to use mucin as a nutrient source (Desai et al., 2016; Sicard et al., 2017). The root mucilage is also a sugar-rich component that serves as a reservoir of nutrients for bacteria (Knee et al., 2001). In many ways, the gut and rhizosphere microbiota have a number of similarities that deserves further research.

Our findings suggest that, in soybean and pea, RET acts not as an impassable barrier, but as a protective filter regulating microorganism behavior around the root tip. It controls the access of microorganisms (pathogenic or beneficial) to the root tip by limiting their density and/or modifying their motility (i.e., speeds and trajectories). It is also possible to consider the RET as a receptacle, or even a micro-niche, for microbial communities whose equilibrium would be finely controlled by the root tip. The RET could thus constitute a microbial reservoir which would facilitate colonization of the rhizosphere as the root grows and develops within the soil.

CRediT authorship contribution statement

Alexia Gaudry: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Magalie Bénard:** Writing – review & editing, Software, Methodology. **Agnès Attard:** Writing – review & editing, Software, Methodology. **Eric Nguema-Ona:** Supervision, Funding acquisition. **Azeddine Driouich:** Writing – review & editing, Validation, Supervision, Investigation, Funding acquisition, Conceptualization. **Barbara Pawlak:** Writing – review & editing, Validation, Supervision, Investigation, Funding acquisition, Conceptualization.

Funding

This work was supported by the Région Normandie and the Centre Mondial de l'Innovation (CM) Roullier.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to thank Florian Barthes (RAGT, Rodez, France) and Gisèle Gleveau (Sem-Partners, Maule, France) for providing us with pea and soybean seeds. We extend our gratitude to Christophe Chamot from the imaging platform (HERACLES, University of Rouen Normandie, France) and Philippe Thomen (University Côte d'Azur, France) for their

valuable discussions and advices.

Data availability

Data will be made available on request.

References

- Ali, M.A., Naveed, M., Mustafa, A., Abbas, A., 2017. The good, the bad, and the ugly of rhizosphere microbiome. In: Kumar, V., Kumar, M., Sharma, S., Prasad, R. (Eds.), *Probiotics and Plant Health*. Springer, Singapore, pp. 253–290. https://doi.org/10.1007/978-981-10-3473-2_11.
- Amicucci, M.J., Galermo, A.G., Guerrero, A., et al., 2019. Strategy for structural elucidation of polysaccharides: elucidation of a maize mucilage that harbors diazotrophic bacteria. *Anal. Chem.* 91, 7254–7265. <https://doi.org/10.1021/acs.analchem.9b00789>.
- Aponte, A., Castillo, O., Cabrera, G., et al., 2017. Rhizobacteria *Pseudomonas fluorescens* and *Azospirillum* sp. association enhances growth of *Lactuca sativa* L. under tropical conditions. *J. Cent. Eur. Agric.* 18, 424–440. <https://doi.org/10.5513/JCEA01/18.2.1916>.
- Attard, A., Gourgues, M., Callemeyn-Torre, N., Keller, H., 2010. The immediate activation of defense responses in *Arabidopsis* roots is not sufficient to prevent *Phytophthora parasitica* infection. *New Phytol.* 187, 449–460. <https://doi.org/10.1111/j.1469-8137.2010.03272.x>.
- Badri, D.V., Vivanco, J.M., 2009. Regulation and function of root exudates. *Plant Cell Environ.* 32, 666–681. <https://doi.org/10.1111/j.1365-3040.2009.01926.x>.
- Bani, M., Cimmino, A., Evidente, A., et al., 2018. Pisatin involvement in the variation of inhibition of *Fusarium oxysporum* f. sp. *pisi* spore germination by root exudates of *Pisum* spp. germplasm. *Plant Pathol.* 67, 1046–1054. <https://doi.org/10.1111/ppa.12813>.
- Bassani, I., Larousse, M., Tran, Q.D., et al., 2020a. *Phytophthora* zoospores: from perception of environmental signals to inoculum formation on the host-root surface. *Comput. Struct. Biotechnol. J.* 18, 3766–3773. <https://doi.org/10.1016/j.csbj.2020.10.045>.
- Bassani, I., Rancurel, C., Pagnotta, S., et al., 2020b. Transcriptomic and ultrastructural signatures of K+-Induced aggregation in *Phytophthora parasitica* zoospores. *Microorganisms* 8, 1012. <https://doi.org/10.3390/microorganisms8071012>.
- Beauregard, P.B., Chai, Y., Vlamakis, H., et al., 2013. *Bacillus subtilis* biofilm induction by plant polysaccharides. *Proc. Natl. Acad. Sci. USA* 110, E1621–E1630. <https://doi.org/10.1073/pnas.1218984110>.
- Berendsen, R.L., Pieterse, C.M.J., Bakker, P.A.H.M., 2012. The rhizosphere microbiome and plant health. *Trends Plant Sci.* 17, 478–486. <https://doi.org/10.1016/j.tplants.2012.04.001>.
- Berne, C., Ellison, C.K., Ducret, A., Brun, Y.V., 2018. Bacterial adhesion at the single-cell level. *Nat. Rev. Microbiol.* 16, 616–627. <https://doi.org/10.1038/s41579-018-0057-5>.
- Bi, S., Sourjik, V., 2018. Stimulus sensing and signal processing in bacterial chemotaxis. *Curr. Opin. Microbiol.* 45, 22–29. <https://doi.org/10.1016/j.mib.2018.02.002>.
- Cannesan, M.A., Durand, C., Burel, C., et al., 2012. Effect of arabinogalactan proteins from the root caps of pea and *Brassica napus* on aphanomyces euteiches zoospore chemotaxis and germination. *Plant Physiology* 159, 1658–1670. <https://doi.org/10.1104/pp.112.198507>.
- Cannesan, M.A., Gangneux, C., Lanoue, A., et al., 2011. Association between border cell responses and localized root infection by pathogenic *Aphanomyces euteiches*. *Ann. Bot.* 108, 459–469. <https://doi.org/10.1093/aob/mcr177>.
- Chaban, B., Coleman, I., Beeby, M., 2018. Evolution of higher torque in *Campylobacter*-Type bacterial flagellar motors. *Sci. Rep.* 8. <https://doi.org/10.1038/s41598-017-18115-1>.
- Chen, L., Liu, Y., 2024. The function of root exudates in the root colonization by beneficial soil rhizobacteria. *Biology* 13, 95. <https://doi.org/10.3390/biology13020095>.
- Cohen, C., Gauci, F.X., Noblin, X., et al., 2023. Kinetics of zoospores approaching a root using a microfluidic device. 2023.06.21.545863. <https://doi.org/10.1101/2023.06.21.545863>.
- Das, B., Nair, G.B., 2019. Homeostasis and dysbiosis of the gut microbiome in health and disease. *J. Biosci.* 44, 117. <https://doi.org/10.1007/s12038-019-9926-y>.
- Desai, M.S., Seekatz, A.M., Koropatkin, N.M., et al., 2016. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* 167, 1339–1353.e21. <https://doi.org/10.1016/j.cell.2016.10.043>.
- Deynze, A.V., Zamora, P., Delaux, P.-M., et al., 2018. Nitrogen fixation in a landrace of maize is supported by a mucilage-associated diazotrophic microbiota. *PLoS Biol.* 16, e2006352. <https://doi.org/10.1371/journal.pbio.2006352>.
- Dodd, C.E.R., 2014. PSEUDOMONAS | introduction. In: Batt, C.A., Tortorello, M.L. (Eds.), *Encyclopedia of Food Microbiology*, second ed. Academic Press, Oxford, pp. 244–247.
- Driouch, A., Follet-Gueye, M.-L., Vicré-Gibouin, M., Hawes, M., 2013. Root border cells and secretions as critical elements in plant host defense. *Curr. Opin. Plant Biol.* 16, 489–495. <https://doi.org/10.1016/j.pbi.2013.06.010>.
- Driouch, A., Gaudry, A., Pawlak, B., Moore, J.P., 2021. Root cap-derived cells and mucilage: a protective network at the root tip. *Protoplasma*. <https://doi.org/10.1007/s00709-021-01660-y>.
- Driouch, A., Smith, C., Ropitiaux, M., et al., 2019. Root extracellular traps versus neutrophil extracellular traps in host defence, a case of functional convergence? *Biol. Rev. Camb. Philos. Soc.* 94, 1685–1700. <https://doi.org/10.1111/brv.12522>.
- Fang, Y., Zhang, L., Jiao, Y., et al., 2016. Tobacco rotated with rapeseed for soil-borne *Phytophthora* pathogen biocontrol: mediated by rapeseed root exudates. *Front. Microbiol.* 7. <https://doi.org/10.3389/fmicb.2016.00894>.
- Fassarella, M., Blaak, E.E., Penders, J., et al., 2021. Gut microbiome stability and resilience: elucidating the response to perturbations in order to modulate gut health. *Gut* 70, 595–605. <https://doi.org/10.1136/gutjnl-2020-321747>.
- Feng, H., Fu, R., Hou, X., et al., 2021. Chemotaxis of beneficial rhizobacteria to root exudates: the first step towards root-microbe rhizosphere interactions. *Int. J. Mol. Sci.* 22, 6655. <https://doi.org/10.3390/ijms22136655>.
- Fortier, M., Lemaitre, V., Gaudry, A., et al., 2023. A fine-tuned defense at the pea root caps: involvement of border cells and arabinogalactan proteins against soilborne diseases. *Front. Plant Sci.* 14. <https://doi.org/10.3389/fpls.2023.1132132>.
- Fourneau, E., Pannier, M., Riah, W., et al., 2024. A “love match” score to compare root exudate attraction and feeding of the plant growth-promoting rhizobacteria *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Azospirillum brasilense*. *Front. Microbiol.* 15. <https://doi.org/10.3389/fmicb.2024.1473099>.
- Furter, M., Sellin, M.E., Hansson, G.C., Hardt, W.-D., 2019. Mucus architecture and near-surface swimming affect distinct *Salmonella typhimurium* infection patterns along the murine intestinal tract. *Cell Rep.* 27, 2665–2678.e3. <https://doi.org/10.1016/j.celrep.2019.04.106>.
- Galiana, E., Cohen, C., Thomen, P., et al., 2019. Guidance of zoospores by potassium gradient sensing mediates aggregation. *J. R. Soc. Interface* 16, 20190367. <https://doi.org/10.1098/rsif.2019.0367>.
- Galiana, E., Fourné, S., Engler, G., 2008. *Phytophthora parasitica* biofilm formation: installation and organization of microcolonies on the surface of a host plant. *Environ. Microbiol.* 10, 2164–2171. <https://doi.org/10.1111/j.1462-2920.2008.01619.x>.
- Galiana, E., Rivière, M.-P., Pagnotta, S., et al., 2005. Plant-induced cell death in the oomycete pathogen *Phytophthora parasitica*. *Cell Microbiol.* 7, 1365–1378. <https://doi.org/10.1111/j.1462-5822.2005.00565.x>.
- Gochauer, M.B., Sealey, L.J., McCully, M.E., 1990. Do detached root-cap cells influence bacteria associated with maize roots? *Plant Cell Environ.* 13, 793–801. <https://doi.org/10.1111/j.1365-3040.1990.tb01095.x>.
- Goldberg, N.P., Hawes, M.C., Stanghellini, M.E., 1989. Specific attraction to and infection of cotton root cap cells by zoospores of *Pythium dissotocum*. *Can. J. Bot.* 67, 1760–1767. <https://doi.org/10.1139/b89-223>.
- Haichar, F. el Z., Santaella, C., Heulin, T., Achouak, W., 2014. Root exudates mediated interactions belowground. *Soil Biol. Biochem.* 77, 69–80. <https://doi.org/10.1016/j.soilbio.2014.06.017>.
- Hassan, M.K., McInroy, J.A., Kloepper, J.W., 2019. The interactions of rhizodeposits with plant growth-promoting rhizobacteria in the rhizosphere: a review. *Agriculture* 9, 142. <https://doi.org/10.3390/agriculture9070142>.
- Hawes, M.C., Bengough, G., Cassab, G., Ponce, G., 2002. Root caps and rhizosphere. *J. Plant Growth Regul.* 21, 352–367. <https://doi.org/10.1007/s00344-002-0035-y>.
- Hawes, M.C., Brigham, L.A., Wen, F., et al., 1998. Function of root border cells in plant health: pioneers in the rhizosphere. *Annu. Rev. Phytopathol.* 36, 311–327. <https://doi.org/10.1146/annurev.phyto.36.1.311>.
- Hawes, M.C., Pueppke, S.G., 1987. Correlation between binding of *Agrobacterium tumefaciens* by root cap cells and susceptibility of plants to crown gall. *Plant Cell Rep.* 6, 287–290. <https://doi.org/10.1007/BF00272000>.
- Hawes, M.C., Smith, L.Y., 1989. Requirement for chemotaxis in pathogenicity of *Agrobacterium tumefaciens* on roots of soil-grown pea plants. *J. Bacteriol.* 171, 5668–5671. <https://doi.org/10.1128/jb.171.10.5668-5671.1989>.
- Hua, C., Wang, Y., Zheng, X., et al., 2008. A *Phytophthora* sojae G-protein α subunit is involved in chemotaxis to soybean isoflavones. *Eukaryot. Cell* 7, 2133–2140. <https://doi.org/10.1128/ec.00286-08>.
- Ito, T., Miyaji, T., Nakagawa, T., Tomizuka, N., 2007. Degradation of dimethyl disulfide by *Pseudomonas fluorescens* strain 76. *Biosci., Biotechnol., Biochem.* 71, 366–370. <https://doi.org/10.1271/bbb.60295>.
- Johansson, M.E.V., Ambort, D., Pelaseyed, T., et al., 2011. Composition and functional role of the mucus layers in the intestine. *Cell. Mol. Life Sci.* 68, 3635–3641. <https://doi.org/10.1007/s00018-011-0822-3>.
- Johansson, M.E.V., Hansson, G.C., 2016. Immunological aspects of intestinal mucus and mucins. *Nat. Rev. Immunol.* 16, 639–649. <https://doi.org/10.1038/nri.2016.88>.
- Kasteel, M., Ketelaar, T., Govers, F., 2023. Fatal attraction: how *Phytophthora* zoospores find their host. *Semin. Cell Dev. Biol.* <https://doi.org/10.1016/j.semcdb.2023.01.014>.
- Knee, E.M., Gong, F.-C., Gao, M., et al., 2001. Root mucilage from pea and its utilization by rhizosphere bacteria as a sole carbon source. *MPMI (Mol. Plant-Microbe Interact.)* 14, 775–784. <https://doi.org/10.1094/MPMI.2001.14.6.775>.
- Laganenka, L., Colin, R., Sourjik, V., 2016. Chemotaxis towards autoinducer 2 mediates autoaggregation in *Escherichia coli*. *Nat. Commun.* 7, 12984. <https://doi.org/10.1038/ncomms12984>.
- Liu, P., Liu, H., Semenc, L., et al., 2022. Length-based separation of *Bacillus subtilis* bacterial populations by viscoelastic microfluidics. *Microsyst. Nanoeng.* 8, 1–11. <https://doi.org/10.1038/s41378-021-00333-3>.
- Lupatelli, C.A., Attard, A., Kuhn, M.-L., et al., 2023. Automated high-content image-based characterization of microorganism behavioral diversity and distribution. *Comput. Struct. Biotechnol. J.* 21, 5640–5649. <https://doi.org/10.1016/j.csbj.2023.10.055>.
- Ma, W., Tang, S., Dengzeng, Z., et al., 2022. Root exudates contribute to belowground ecosystem hotspots: a review. *Front. Microbiol.* 13. <https://doi.org/10.3389/fmicb.2022.937940>.

- Macquet, A., Ralet, M.-C., Kronenberger, J., et al., 2007. In situ, chemical and macromolecular study of the composition of *Arabidopsis thaliana* seed coat mucilage. *Plant Cell Physiol.* 48, 984–999. <https://doi.org/10.1093/pcp/pcm068>.
- Mendes, R., Garbeva, P., Raaijmakers, J.M., 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Rev.* 37, 634–663. <https://doi.org/10.1111/1574-6976.12028>.
- Meng, Y., Zhang, Q., Ding, W., Shan, W., 2014. *Phytophthora parasitica*: a model oomycete plant pathogen. *Mycology* 5, 43–51. <https://doi.org/10.1080/21501203.2014.917734>.
- Meyen, F.J.F., 1837. *Ueber die Secretiones-Organen der Pflanzen*, vol. 99. F.H. Morin, Berlin.
- Mitchell, H.J., Kovac, K.A., Hardham, A.R., 2002. Characterisation of *Phytophthora nicotianae* zoospore and cyst membrane proteins. *Mycol. Res.* 106, 1211–1223. <https://doi.org/10.1017/S0953756202006512>.
- Nazari, M., Bickel, S., Benard, P., et al., 2022. Biogels in soils: plant mucilage as a biofilm matrix that shapes the rhizosphere microbial habitat. *Front. Plant Sci.* 12, 798992. <https://doi.org/10.3389/fpls.2021.798992>.
- Nguyen, T.N.H., Fortier, M., Bernard, S., et al., 2024. Sweet specificities of the root extracellular trap of perennial ryegrass (*Lolium perenne*), a fructan accumulating plant. *Environ. Exp. Bot.* 222, 105743. <https://doi.org/10.1016/j.envexpbot.2024.105743>.
- Okumura, R., Kurakawa, T., Nakano, T., et al., 2016. Lypd8 promotes the segregation of flagellated microbiota and colonic epithelia. *Nature* 532, 117–121. <https://doi.org/10.1038/nature17406>.
- Pang, Z., Mao, X., Zhou, S., et al., 2023. Microbiota-mediated nitrogen fixation and microhabitat homeostasis in aerial root-mucilage. *Microbiome* 11, 85. <https://doi.org/10.1186/s40168-023-01525-x>.
- Piggot, P., 2009. *Bacillus subtilis*. *Bacteria* 46–56. <https://doi.org/10.1016/B978-012373944-5.00036-5>.
- Ropitiaux, M., Bernard, S., Follet-Gueye, M.-L., et al., 2019. Xyloglucan and cellulose form molecular cross-bridges connecting root border cells in pea (*Pisum sativum*). *Plant Physiol. Biochem.* 139, 191–196. <https://doi.org/10.1016/j.plaphy.2019.03.023>.
- Ropitiaux, M., Bernard, S., Schapman, D., et al., 2020. Root border cells and mucilage secretions of soybean, *Glycine max* (merr) L.: characterization and role in interactions with the oomycete *Phytophthora parasitica*. *Cells* 9. <https://doi.org/10.3390/cells9102215>.
- Rovira, A.D., 1969. Plant root exudates. *Bot. Rev.* 35, 35–57. <https://doi.org/10.1007/BF02859887>.
- Schroeder, B.O., 2019. Fight them or feed them: how the intestinal mucus layer manages the gut microbiota. *Gastroenterology Report* 7, 3–12. <https://doi.org/10.1093/gastro/goy052>.
- Schwanbeck, J., Oehmig, I., Groß, U., et al., 2021. Clostridioides difficile single cell swimming strategy: a novel motility pattern regulated by viscoelastic properties of the environment. *Front. Microbiol.* 12, 715220. <https://doi.org/10.3389/fmicb.2021.715220>.
- Shirakawa, M., Matsushita, N., Fukuda, K., 2023. Visualization of root extracellular traps in an ectomycorrhizal woody plant (*Pinus densiflora*) and their interactions with root-associated bacteria. *Planta* 258, 112. <https://doi.org/10.1007/s00425-023-04274-1>.
- Sicard, J.-F., Le Bihan, G., Vogeleer, P., et al., 2017. Interactions of intestinal bacteria with components of the intestinal mucus. *Front. Cell. Infect. Microbiol.* 7. <https://doi.org/10.3389/fcimb.2017.00387>.
- Sugiyama, A., 2019. The soybean rhizosphere: metabolites, microbes, and beyond—a review. *J. Adv. Res.* 19, 67–73. <https://doi.org/10.1016/j.jare.2019.03.005>.
- Tan, M.S., Rahman, S., Dykes, G.A., 2016. Pectin and xyloglucan influence the attachment of *Salmonella enterica* and *Listeria monocytogenes* to bacterial cellulose-derived plant cell wall models. *Appl. Environ. Microbiol.* 82, 680–688. <https://doi.org/10.1128/AEM.02609-15>.
- Tian, T., Reverdy, A., She, Q., et al., 2020. The role of rhizodeposits in shaping rhizomicrobiome. *Environmental Microbiology Reports* 12, 160–172. <https://doi.org/10.1111/1758-2229.12816>.
- Tran, Q.D., Galiana, E., Thomen, P., et al., 2022. Coordination of two opposite flagella allows high-speed swimming and active turning of individual zoospores. *Elife* 11, e71227. <https://doi.org/10.7554/eLife.71227>.
- Tran, T.M., MacIntyre, A., Hawes, M., Allen, C., 2016. Escaping underground nets: extracellular DNases degrade plant extracellular traps and contribute to virulence of the plant pathogenic bacterium *Ralstonia solanacearum*. *PLoS Pathog.* 12, e1005686. <https://doi.org/10.1371/journal.ppat.1005686>.
- Valibeknejad, M., Abdoli, S.M., Alizadeh, R., et al., 2023. Insights into transport in mucus barrier: exploring particle penetration through the intestinal mucus layer. *J. Drug Deliv. Sci. Technol.* 86, 104752. <https://doi.org/10.1016/j.jddst.2023.104752>.
- Vives-Peris, V., de Ollas, C., Gómez-Cadenas, A., Pérez-Clemente, R.M., 2020. Root exudates: from plant to rhizosphere and beyond. *Plant Cell Rep.* 39, 3–17. <https://doi.org/10.1007/s00299-019-02447-5>.
- Voiniciuc, C., Schmidt, M.H.-W., Berger, A., et al., 2015. MUCILAGE-RELATED10 produces galactoglucomannan that maintains pectin and cellulose architecture in *Arabidopsis* seed mucilage. *Plant Physiology* 169, 403–420. <https://doi.org/10.1104/pp.15.00851>.
- Wen, F., VanEtten, H.D., Tsapralis, G., Hawes, M.C., 2007. Extracellular proteins in pea root tip and border cell exudates. *Plant Physiol.* 143, 773–783. <https://doi.org/10.1104/pp.106.091637>.
- Wen, F., White, G.J., VanEtten, H.D., et al., 2009. Extracellular DNA is required for root tip resistance to fungal infection. *Plant Physiol.* 151, 820–829. <https://doi.org/10.1104/pp.109.142067>.
- Wong-Ng, J., Celani, A., Vergassola, M., 2018. Exploring the function of bacterial chemotaxis. *Curr. Opin. Microbiol.* 45, 16–21. <https://doi.org/10.1016/j.mib.2018.01.010>.
- Yadav, A., Saini, I., Kaushik, P., et al., 2021. Effects of arbuscular mycorrhizal fungi and P-solubilizing *Pseudomonas fluorescens* (ATCC-17400) on morphological traits and mineral content of sesame. *Saudi J. Biol. Sci.* 28, 2649–2654. <https://doi.org/10.1016/j.sjbs.2021.03.024>.
- Yu, C., Liu, X., Zhang, X., et al., 2021. Mycosubtilin produced by *Bacillus subtilis* ATCC6633 inhibits growth and mycotoxin biosynthesis of *Fusarium graminearum* and *Fusarium verticillioides*. *Toxins* 13, 791. <https://doi.org/10.3390/toxins13110791>.