AAPG2023	DivIDE		PRCI
Coordonné par :	Philippe VANDENKOORNHUYSE	36 months	Aide totale demandée ANR
CE20-A3 Biologie des animaux, des organismes photosynthétiques et des microorganismes LS08-12 Microbial ecology and evolution			

Division of labour among genetically Identical bacterial intra-populations as an organisational rule Driving root microbiota Establishment (DivIDE)

Tableau récapitulatif des personnes impliquées dans le projet / Summary table of people involved in the project

Country	University or Institution	Last Name	First Name	Current position	Role in the project	Involvement (person.months)
France	*University of Rennes, UMR6553 ECOBIO*	*Vandenkoornhuyse*	*Philippe*	*Professor*	*Scientific coordinator WP2 leader*	*12.6*
		xxx	*xxx*	*PostDoc to be hired**	*Shared postdoc WP2 primarily, WP1 & WP3*	*32*
France	*University of Rennes, UAR3343 OSUR*	*Michon-Coudouel*	*Sophie*	*Engineer*	*Molecular biology and omics support (WP2 & WP3)*	*3*
		Causse-Védrines	*Romain*	*Engineer*	*Molecular biology and omics support (WP2 & WP3)*	*3*
		Peigne	*Antoine*	*Engineer*	*Single-Cell omics support (WP2 & WP3)*	*6*
		xxx	*xxx*	*Molecular Biology Engineer to be hired**	*Single Cell omics (WP2)*	*6*
France	*INRAE, UMR IGEPP*	*Vannier*	*Nathan*	*Full researcher*	*WP1 leader*	*7.2*
		xxx	*xxx*	*2 x MSc Internship to be hired**		*2 x 6*
Germany	*Max Planck Institute for Plant Breeding Research, Cologne, Germany*	*Hacquard*	*Stephane*	*Group leader*	*WP3 leader*	*7.2*
		XXX	*XXX*	*PhD to be hired **	*WP1 & WP3*	*36*

**** To be hired in the frame of this project***

Evolution(s) éventuelle(s) de la proposition détaillée par rapport à la pré-proposition ou à l'enregistrement (PRCI) / Possible evolution(s) of the full proposal compared to the pre-proposal or registration (PRCI)

Since the registration of this PRCI project, the scientific and technological objectives and the composition of the consortium did not change but the amount of aid requested for the proposal has

increased because we had not included the overheads within the first budget estimates. At the stage of PRCI registration the web interface did not contain an automatic overhead calculator (~13%) and we had omitted this aspect.

I. Contexte, positionnement et objectif(s) de la proposition / Context, positioning and objective(s) of the proposal

a. Objectifs et hypothèses de recherche / Research objectives and hypotheses

Context of the proposal

Roots of healthy plants host diverse bacteria that are collectively referred to as the bacterial root microbiota. Unlike their multicellular eukaryotic hosts that evolved diverse cell-types to achieve distinct biological functions and promote a division of labour, unicellular organisms such as bacteria rely on metabolic exchange(s) with their surrounding biotic environment. Recent reports, including our own work (Mataigne et al. 2021, Mataigne et al. 2022), indicate that metabolic interdependencies and cross feeding exchanges are widespread among taxonomically diverse bacteria and likely drive microbial co-existence within complex bacterial communities (e.g. Estrella et al., 2016; Adkins-Jablonsky et al., 2021).

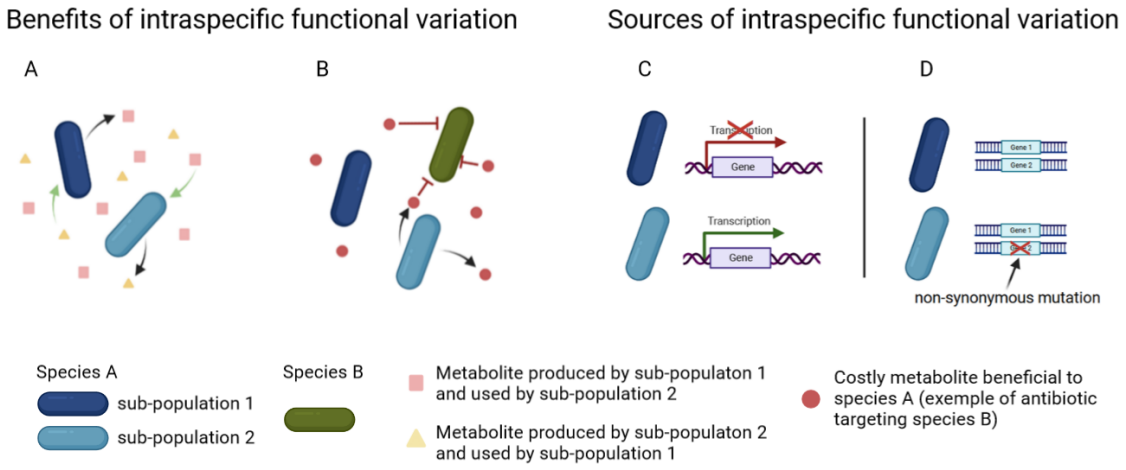


Figure 1. Examples of benefits and sources of intraspecific division of labour between intra-populations based on metabolic complementation and costly "common good" metabolite production. From the left to the right, co-metabolism within an isogenic bacterial population (A) and production of antimicrobials targeting other bacteria (B) can be considered as division of labour in a population. These mechanisms of division of labour can be based on differential transcription between bacteria (C) within a population and/or genetic variations between intra-populations (D).

However, a major unsolved question is whether populations of genetically identical bacteria can minimise energetically costly processes by each executing different metabolic tasks at the intra-population level. Here, we hypothesise that metabolic cooperation between bacterial intra-populations plays a key role in modulating population dynamics, competitiveness and persistence at the root soil-interface. This hypothesis also builds on the idea that within a population, bacteria are inclined to 'noisy regulation' of metabolism (Lopez & Wingreen, 2022), i.e. they do not all adjust their genome expression to the environmental constraints in the same way. In a stable environment, this would be expected to limit bacterial growth. However, because bacteria excrete

compounds ('leaky function' forming a 'metabolic marketplace'), the selection pressures at the population level would favour intra-population cross-feeding(s) fitting to the constraints, either supported by variations in gene expression or/and selection for useful variants that arise from non-synonymous mutations (**Figure 1**). Thus, by 'noise-averaging cooperation' (Lopez & Wingreen, 2022) an intra-population-level division of labour is expected.

Successful establishment of bacteria at roots is driven by multiple independent biological processes involved in both host-microbe (i.e., signal recognition, chemotaxis, surface attachment, biofilm formation, virulence factors) and microbe-microbe interactions (i.e., production of antimicrobials or public goods) (Knights et al. 2021). Therefore, we postulate that the simultaneous activation of these diverse processes is costly and that cooperation between genetically identical strains is key for promoting bacterial pervasiveness at roots. Consistent with this hypothesis, we recently demonstrated that the robust root coloniser P*seudomonas brassicacearum* R401 (hereafter referred to as *PsR401*) deploys multiple independent strategies that co-function to promote colonisation and persistence at roots (Getzke et al., 2023). Notably, we identified two independent processes involved in microbe-microbe competition – namely the production of an antimicrobial and of a molecule scavenging the micronutrient iron – that act additively to promote strain competitiveness at roots (Getzke et al., 2023). We identified a third bacterial locus in *Ps*R401 involved in the production of a phytotoxin that promotes both pathogenicity and root colonisation in mono-association experiments with *A. thaliana* (unpublished, see below). Our work provides proof-of-concept data illustrating that *Ps*R401 deploys diverse exo-metabolites during root microbiota establishment that have versatile functions in host-microbe and microbe-microbe interactions. Together with earlier work (Gu et al. 2020, Harbort et al 2020), it also delineates iron as a major micronutrient modulating strain competitiveness and proliferation at roots. Given that the public good iron becomes rate-limiting in the root compartment and that production of the above-mentioned processes are all modulated by iron availability (Lim et al., 2012; Palma et al., 2003, Mo et al. 1991), we anticipate that division of labour between bacterial intra-populations is bolstered under iron limiting conditions such as those found in the root habitat.

Objectives and hypotheses of the proposal

The major goal of the project is to obtain mechanistic insights into whether and how a robust bacterial root coloniser can partition tasks within intra-populations to achieve greater functional diversity, minimise the energetic cost of processes, and adapt to environmental constraints to promote population density at roots. Given that at a micro-scale, niche partitioning is often observed between bacterial populations at roots, we hypothesise that cooperation within a bacterial population is at least as important as cooperation between bacterial populations for promoting overall population density. We anticipate that a single cell can only achieve a limited number of functions and that greater functional diversity can be achieved through within-population cooperation, especially under nutritional constraints. Given that iron is rate-limiting in roots, we particularly aim at testing the relevance of division of labour between bacterial intra-populations in the context of iron availability. The project objectives are summarised in three key hypotheses (**H1**-**H3**), corresponding to three separate work packages (**WPs**), which are described in detail below in the section C ' and **Figure 2**.

H1 (WP1): Subtle genetic perturbations in *Pseudomonas brassicacearum* (*PsR401*) mutants modulate population dynamics *in vitro*.

H2 (WP2): In a population of *Pseudomonas brassicacearum* (*PsR401*), individuals activate different biological and metabolic processes, promoting intra-population functional diversity and allowing a rapid division of labour to colonise the environment (with a particular focus on the root-environment)

H3 (WP3): Population dynamics and density of *Pseudomonas brassicacearum* (*PsR401*) at roots is modulated by cooperation between bacterial intra-populations.

Figure 2. Description of work packages to characterise division of labour in bacteria intra-populations. From the left to the right, in WP1 we will take advantage of a transposon insertion mutant library to investigate how subtle genetic perturbations affect population density of mixtures of WT and mutant *PsR401* strains to identify genes promoting both intra-populations' density and thus overall population growth. In WP2 we will investigate, from microbial single cell transcriptomes of *PsR401* populations subjected to environmental constraints, whether metabolic cooperation and/or noisy regulation can promote growth at the population level. In WP3 we will design reporter lines for major genes affecting *PsR401* growth in roots to investigate how these costly functions are regulated at the population level in time and in space and whether *PsR401* partition these tasks at the population level.

b. Positionnement par rapport à l'état de l'art / Positioning in relation to the state of the art

DivIDE has the ambition to define a novel research line in the plant microbiota research field and to identify multiple mechanisms that promote functional diversity in a genetically identical bacterial population. By combining strain engineering methods, single cell omics, microbiota reconstitution experiments in gnotobiotic plant systems, as well as high-resolution real-time confocal

microscopy, DivIDE will generate important mechanistic insights explaining emergence and maintenance of cooperative traits within a bacterial population.

By testing whether task partitioning and division of labour occur between bacterial intra-populations in a core lineage of the bacterial root microbiota, DivIDE also aims at identifying multiple points of control by which bacterial density can be 'regulated' through metabolic exchange between bacterial cells. Despite its high degree of innovation, the project builds upon very recently acquired knowledge (Getzke et al. 2023) and has broad ramifications to understand the rules driving microbiota establishment and host niche colonisation, beyond the plant-microbe interaction research field. Because DivIDE aims at investigating bacterial cooperative processes at single-cell (i.e. WP2) and spatialized (i.e. WP3) resolutions, it will generate novel knowledge on the influence of spatiotemporal dynamics of bacterial populations over the induction of cooperative traits at roots.

Cooperative interactions have been extensively described between phylogenetically unrelated bacterial species (e.g. Zelezniak et al. 2015). Here, we reason that in a spatial context, cooperation is more likely to occur between intra-populations of genetically identical bacteria than between genetically distant strains with limited niche overlap. Therefore, we predict that evolution of interdependencies between microbes involves both between-population and within-population functional heterogeneity.

c. Méthodologie et gestion des risques/Methodology and risk management

Organisms must carry out a variety of functions in order to live and reproduce. But they are constrained in how much energy they can devote to each of these various processes, leading to trade-offs. Specialisation in certain traits and cooperation with other organisms that can assist by contributing extra, complementary functions are possible ways to cope with this problem. Both parties profit from the interaction by the reciprocal exchange of metabolites and/or services. This functional specialisation, also known as the division of labour, is a phenomenon that occurs frequently in nature and at all levels of biological organisation. In this context, the project is organised in 3 complementary Work Packages (**Figure 2** and below, WPs description) addressing the working hypotheses H1, H2 and H3 respectively.

WP1: Subtle genetic perturbations in *PsR401* support division of labour, promoting population density under iron limitations. (WP coordinator : N. Vannier, INRAE)

Targeted scientific purpose of the WP:

The aim of this WP is to test hypothesis 1, namely that subtle genetic variations in intra-population provide a basis for division of labour by task partitioning at the intra-population level, promoting overall population growth. In this aim we will take advantage of an existing transposon insertion mutants library of *PsR401* to mimic transcriptional heterogeneity in *PsR401* and will screen for mutants promoting the growth of the WT strain and the overall population. Beyond testing whether subtle genetic perturbations in *PsR401* mutants can positively or negatively impact *PsR401* WT intra-population dynamics and overall population growth, this approach will also identify genes and functions promoting population density through bacteria-bacteria cooperation. We therefore anticipate that gene inactivation in some mutants will have a cascading effect on the fitness of the WT intra-population and the overall population density.

How ?

Preliminary work in relation to the WP:

We propose to mimic transcriptional heterogeneity in *PsR401* using > 6,000 insertion mutants derived from random *tn5* mutant library that we have recently constructed (Getzke et al., 2023). Taking

advantage of this library as a reservoir of genetic perturbations that can support division of labour between intra-populations, we will assemble different populations and monitor their performance in the context of limited iron availability. We also previously labelled PsR401 with a GFP promoter allowing to differentially monitor the growth of the WT strain (i.e. with green fluorescence) even in the presence of single-gene insertion mutants. Building on these preliminary developments and using high throughput *in vitro* assays we will test the extent to which subtle genetic perturbations in a mutant can modulate the growth of the focal GFP-labelled *PsR401* WT strain, and the overall population density. Because it is likely that metabolic complementations and other task partitioning mechanisms are especially important under nutrient limitations, we will investigate intra-population division of labour under nutrient depleted conditions (iron as a limiting nutrient).

Anticipated experimental plan to identify genetic perturbations promoting division of labour in bacterial intra-populations

In a first step, we aim at identifying single gene mutants promoting population density. We will first measure the mutants' growth with a plate reader (OD600, 6000 growth curves) to select the mutants that retained the growth dynamic of the WT *PsR401* (see protocol steps in **Figure 3**). The selected subset of *tn5* mutants will then be used for 'one on one' interaction assays with the GFP-labelled WT. We will co-inoculate the GFP-labelled WT with each mutant individually in liquid culture medium with reduced iron content (estimated number of assays ~5000). We will use the WT *PsR401* as a reference and will measure OD600 to estimate the density of the overall population (i.e. WT + single mutant). Because the WT is GFP-labelled we will measure green fluorescence and therefore differentiate the WT density from the mutant density. Knowing both the overall population and the WT density we will be able to calculate the mutant density. This process will identify associations for which the WT and/or the overall population growth is higher than the WT population alone.

In a second step, the candidate mutants improving population density will be confirmed by repeating the previous assay 3 additional times to confirm the observed phenotype (estimated number of assays ~1000). We will then identify the insertion site of the transposon with PCR amplification and SANGER sequencing of the amplified fragment. We will validate the importance of the identified genes independently by generating knock-out mutants, ensuring the observed phenotype is not method dependent. This will provide insight into whether subtle genetic perturbations could promote overall population density because they underpin the evolution of division of labour between intra-populations.

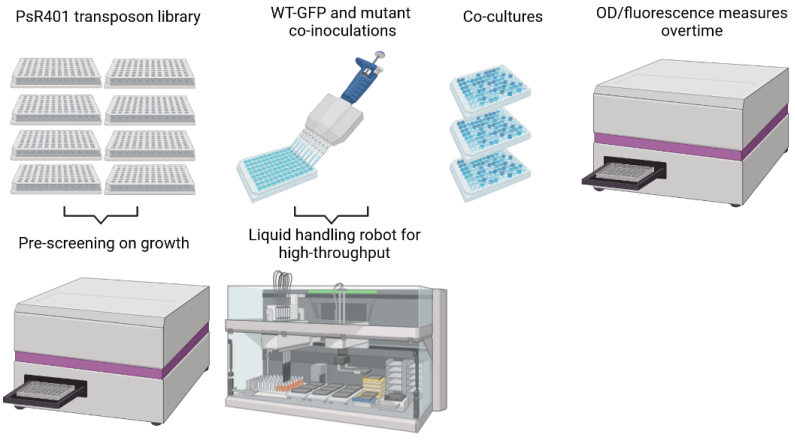


Figure 3: Anticipated protocol to screen the transposon library for functions underlying division of labour in *PsR401*. To maximise screening ability, all one on one interactions will be done in 96 well plates and population density will be measured with a plate reader. The 5000 interactions will represent a large effort of liquid handling; the use of a liquid handling robot is anticipated to facilitate this step.

In a third step, we will combine different mutants for which a density promotion has been observed to test whether intra-population

diversity promotes division of labour under nutrient limitations (i.e. additive and synergistic effects of genetic perturbations). To do so, we will construct populations with variable intra-population richness (assembly of single-gene mutants) and monitor the population density under iron limitation (estimated number of assays ~1000).

Finally, the identified genes and functions promoting division of labour at the population level will inform WP2 and WP3 to monitor single-cell expression and visualise this expression in roots and bacterial communities.

WP2: Division of labour at intra-population level of *PsR401* by bacterial single cell transcriptomics analysis. (WP coordinator : P Vandenkoornhuyse, Université de Rennes, UMR 6553 ECOBIO)

Targeted scientific purpose of the WP:

The aim of this WP is to test hypothesis 2, the division of labour within a microbial isogenic population of *PsR401*, either by metabolic specialisation of intra-populations or by 'noise-averaging cooperation' from 'noisy regulation' of metabolism (Lopez & Wingreen, 2022). In this aim, we will develop an innovative pipeline of bacterial single cells transcriptomics analysis (mscRNAseq) to monitor the genome expression from single isolated cells of *PsR401*.

How ?

Preliminary work in relation to the WP: We have recently developed a microbial single cell whole genome sequencing strategy (mscWGS, publication in preparation, Fig 4) that allows to (i) isolate individual living bacterial cells using a cellenONE instrument, to (ii) use a proven compatible bacterial lysis protocol and (iii) build upon the proof of concept of Eukaryotic single cell whole RNAs sequencing method (Cellenion).

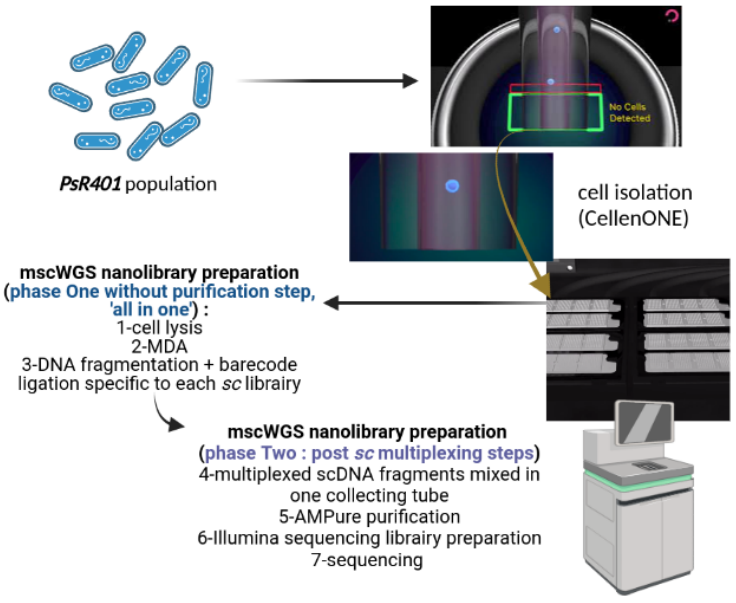


Figure 4 : **Validated bacterial single cell whole genome sequencing wetlab pipeline.** After isolation of bacterial single cells (cellenONE), each single cell is first lysed and then the genome is exponentially amplified (MDA), fragmented, end-repaired and a specific multiplex tag is ligated on each fragment of a given single cell library. All these steps are performed without any purification step having validated the buffer's compatibility (absence of enzyme inhibition). The phase Two of the mscWGS library construction is more classical. This mcsWGS is the backbone for the adaptation of a mscRNA seq library construction (see Figure 5).

Anticipated protocol to perform the bacterial single cells transcriptomics analysis. Single cell RNA seq (transcriptomic analysis of single cells) has been developed to successfully analyse the heterogeneity of genome expression in eukaryotic cells (principally humans and other mammals, e.g. Papalexi & Satija 2018) by taking advantage of the existence of a poly(A) stretch at the end of the neo-synthesized mRNA. In bacterial RNA, such poly(A) stretch does not exist, but poly(A)-independent single cell RNA seq methods have recently been developed (Blattman et al., 2020; Imadahl et al., 2020; Kushina et al., 2021). Alternatively, a poly(A)-transformation of RNA

molecules after RNA fragmentation and tailing has also been demonstrated to be efficient (i.e. VASAseq in Salmen et al., 2022). These 2 alternative strategies can be adapted to the nanolibrary preparation of mscRNA seq. Given the number of steps and complexity of the different poly(A)-independent single cell RNA seq strategies, we think that the VASAseq strategy (Figure 5) is easier to adapt. In short, tests of lysis buffer compatibility with ligation and end-repair steps performed allows 2 alternative choices (work developed in 2021-2022 within the project ANR LABCOM 'microscale-lab'). The poly(A) tailing is mandatory and this step will avoid the subsequent recruitment of genomic DNA fragments. After the indexing PCR (amplification of the constructed fragment containing the 2 adapters at extremities) (Figure 5), all the fragments of each single cell library will be collected in one tube. Before sequencing,

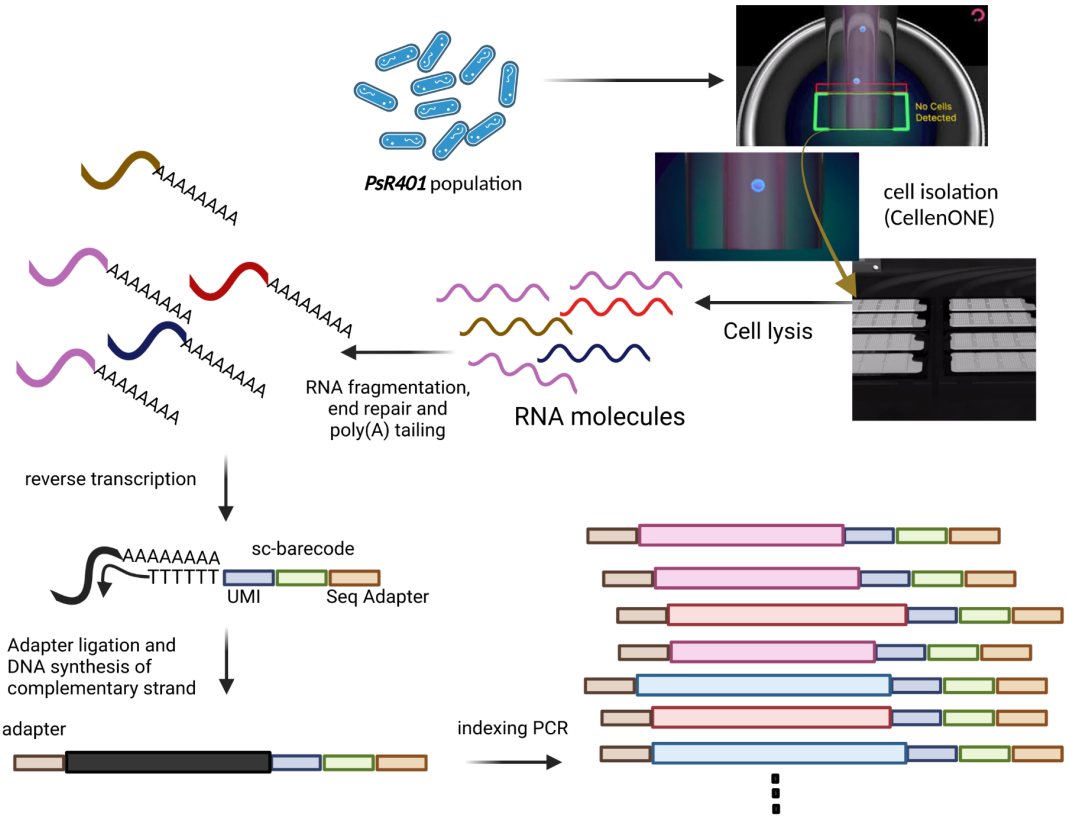


Figure 5 : Anticipated backbone protocol for microbial single cell RNA seq (mscRNAseq). Cell isolation and cell lysis will be performed on the basis of a validated previous work. For the next step the need to fragment RNAs will be evaluated, end repair and poly A tailing is mandatory. From this poly(A) we will use synthesised poly(T) with a tail containing Unique Molecular Identifiers (UMI), a single cell barcode and the sequencing adapter. After the reverse transcription from the hybridised poly(T), an adapter will be ligated to the extremity to end with a double strand molecule. Before sequencing a final indexing PCR will amplify the constructed fragments containing the 2 adaptors at the extremities.

an additional step of rRNA depletion will be performed. The advantage of including a unique molecule identifier (UMI, Figure 5) within the library construction is to normalise differences of amplification among constructed fragments and more importantly to have an estimation of the quantity of a given RNA we have for a given *PsR401* single cell.

The protocol that will be developed in this WP will be used (task 1) to analyse a *PsR401* WT grown under constant constrained growth conditions under limited iron concentration in a chemostat to assess the expression heterogeneity among cells and at the population level. This will allow us to test

in a simple context if division of labour occurs by 'noise-averaging cooperation', i.e. differential genome expression among cells, or division of labour resulting from specialised expression profiles at a intra-population level (Figure 5, 1000 cells analysed by mscRNAseq). This analysis will be combined with *PsR401* single cell whole genome sequencing (Figure 4, 1000 cells analysed by mscWGS) to assess the possible genetic variation that can arise *de novo* during the population growth. We will also analyse (task 2), in connexion to WP1, combinations of *PsR401* WT and mutant displaying either positive or negative impact on the overall population growth to test for differential expression among cells and to determine how mutations could lead to division of labour (i.e. intra-populations organised by both mutation and nested specialised expression profiles) (at least 2 combinations with 1000 mscRNAseq for each). The transcriptomic data produced will be analysed with state of the art tools. Briefly, the sequencing reads will be mapped against PsR401 high-quality reference genome using STAR aligner (Dobin et al., 2013), transcriptomes will be visualised using t-SNE (distributed stochastic neighbour embedding) and further differential expression analysis will be done with single-cell RNA-seq specific tools like SCANPY (wolf et al., 2018 ; Luecken et al., 2019; Stuart et al., 2019).

The various steps of WP2 will be implemented in the EcogenO platform (UAR3343 OSUR), member of the Research Infrastructure AnaEE-france and AnaEE-ERIC.

WP3 : Population dynamics and density of *Pseudomonas brassicacearum PsR401* at roots is modulated by cooperation between bacterial intra-populations. (WP coordinator : S. Hacquard, MPI Cologne)

Targeted scientific purpose of the WP:

The aim of this WP is to test hypothesis 3, namely that energetically-costly bacterial molecules important for root colonisation and competition with microbiota members, are likely not all concomitantly and homogeneously activated at roots in a bacterial population and that bacterial intra-populations likely coordinate activation of these processes to divide biological tasks in a cost effective manner. Using *PsR401* as a genetically tractable system, we propose to test whether the production of three energetically-costly and specialised exometabolites – an antimicrobial, an iron chelating molecule, and a phytotoxin – is partitioned between *Ps*R401 intra-populations during root microbiota establishment to promote strain establishment, dominance, and persistence at roots.

How ?

Preliminary work in relation to the WP:

We have recently identified three biosynthetic gene clusters in this robust bacterial root coloniser that are involved in the production of the antimicrobial 2,4-diacetylphloroglcucinol, the iron chelating molecule Pyoverdine and the phytotoxin Syringopeptin (**Figure 6**). We demonstrated that the two first molecules are involved in bacteria-bacteria competition at roots and co-function not only as root competence determinants but also as drivers of bacterial microbiota assembly (Getzke et al. 2023) (**Figure 6a,b**). In contrast, the last exo-metabolite has limited relevance for microbe-microbe competition but represents a pathogenicity and root colonisation determinant. Remarkably, inactivation of the production of this metabolite was sufficient to turn this opportunistic pathogen into a beneficial isolate under salt stress (**Figure 6a,c**). Our preliminary data provide proof-of-concept data indicating that *Ps*R401 deploys at least three unrelated exo-metabolites that co-function together as root competence determinants that drive successful establishment of this robust root coloniser.

Anticipated experimental plan to visualise activation of these processes at single-cell resolution during root microbiota establishment.

In a first step, we will generate three independent *PsR401* promoter reporter lines (P*phlD::*eGFP, P*pvdY::*mCerulean*, and* P*sypC::*mCherry*)* using the low copy plasmid pSEVA221 containing the promoter::fluorophore constructs. Promoter regions will be amplified from *PsR401* genomic DNA whereas *eGFP*, *mCerulean*, and *mCherry* will be amplified from previously published plasmid stocks. This vector will be integrated into *E. coli* BW29427 cells and subsequently integrated into *PsR401* via biparental conjugation. Using this strategy, we will test promoter leakiness and assess whether the induction observed reflects the expected induction observed in a native context. After this validation step, we will generate a three-colour promoter reporter strain *PsR401* (P*phlD::*eGFP-P*pvdY::*mCerulean-P*sypC::*mCherry) to study activation of these processes at single-cell resolution and to test whether they are activated heterogeneously by different bacterial intra-populations during root colonisation. In this reporter strain, the expression eGFP, mCerulean, or mCherry will be driven by the previously validated promoter regions. Because we aim at chromosomal integration of the plasmid, the construct will be flanked by homologous recombination sites that drive integration within a non-coding genomic region of *PsR401*, as previously done in our group. The respective PCR products will be assembled via Gibson assembly into the pk18mobsac plasmid. This vector will be integrated into *E. coli* BW29427 cells and subsequently integrated into *PsR401* via biparental conjugation. After selection of the transformants on Kanamycin-containing media, we will validate plasmid integration and select plasmid-free transformants using a sacB counter selection strategy. We will adapt our protocol according to recently published work describing the construction of such three-colour reporter strains (Han et al. 2019, Brameyer et al. 2022).

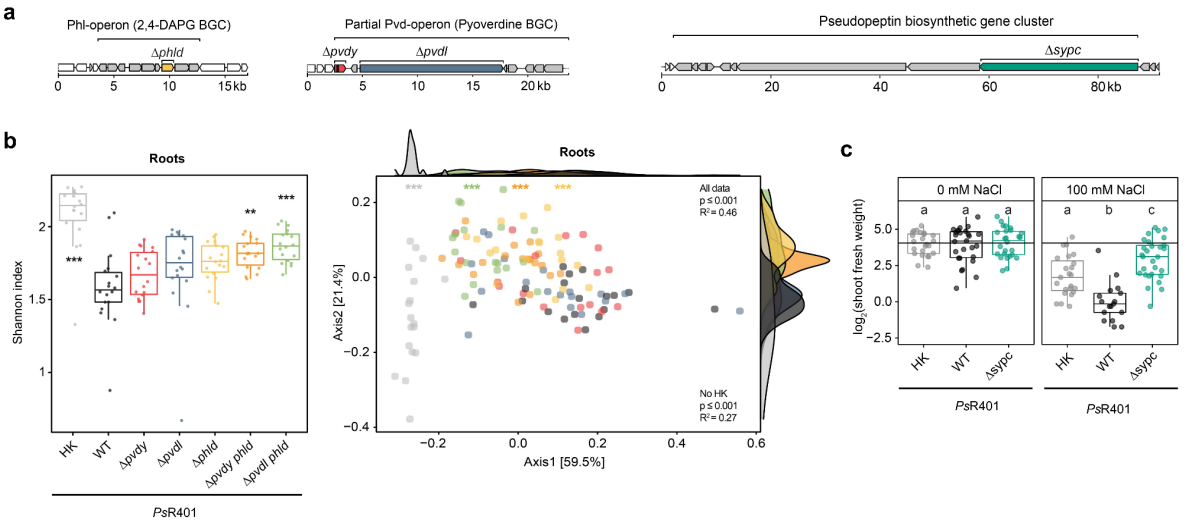


Figure. 6: Three *Ps*R401 operons involved in microbe-microbe-host interactions. a) Schematic view of the three biosynthetic gene clusters (BGCs) involved in the production of 2,4-diacetylphloroglucinol (DAPG), pyoverdine, and syringopeptin. Genes highlighted in colours correspond to the R401 mutant strains already generated. **b)** Diversity (left) and composition (right) of an 18-member bacterial SynCom (*A. thaliana* root samples, FlowPot gnotobiotic system) in the presence of *PsR401* wild-type (WT) or mutant strains impaired in the production of pyoverdine (*Δpvdy, Δpvdl*), DAPG (*Δphld*), or both (*Δpvdy Δphld, Δpvdl Δphld*). HK: heat-killed *PsR401* (See Getzke et al. PNAS, 2023). **c**) *A. thaliana* shoot fresh weight in mono-association experiments with *Ps*R401 HK, WT, or a mutant strain impaired in syringopeptin production (*Δsypc)* under 0 mM and 100 mM NaCl *(*FlowPot gnotobiotic system, unpublished). Note that inactivation of this single gene turns this opportunistic pathogen into a beneficial growth-promoting strain under salt stress.

In a second step, we aim at visualising induction of these processes in time and in space at single cell resolution during root colonisation using the super-resolution Airyscan2 LSM980 inverted confocal imaging system. Roots of germ-free *Arabidopsis thaliana* will be recolonized by the individual reporter strains and/or by the three-colour reporter strain using a transparent soil system that was previously shown to better maintain root architecture of soil-grown plants than agar based systems (Ma et al. 2019). Activation of these processes will be first monitored in real time during *Ps*R401 root colonisation for at least 5 days. Based on our recent work (Getzke et al., 2023), activation of these processes is expected to be modulated by the presence of bacterial competitors and by iron levels at the root interface. We will recolonize our transparent soil with a low-diversity synthetic bacterial community (SynCom) of 18 strains recently used in combination with *PsR401* (Getzke et al., 2023) and apply different gradients of $FeCl_3$ in the system. Using this modular strategy, we will test whether combined abiotic (iron limitation) and biotic (presence of bacterial competitors) factors promote division of labour between *PsR401* intra-populations. These perturbation experiments in strictly controlled laboratory conditions will allow us to assess the extent to which processes linked to root colonisation, competition with bacteria, and iron starvation are activated at single cell resolution to promote *PsR401* dominance at roots. We anticipate that these multiple processes cannot be induced simultaneously by a single cell, and that expression heterogeneity between cells is likely to explain the pervasiveness of *Pseudomonas* taxa in roots of natural plant populations (Thiergart et al. 2019).

Links in between WPs:
-Choice of the same *Pseudomonas brassicacearum* R401 (*PsR401*)
-Concentration of available Iron limited to develop an new knowledges regarding the costly pyoverdine synthesis, i.e. to have a particular focus about division of labour regarding gene expression for the pyoverdine synthesis (link with WP3)
-In WP2, combinations of co-cultivated *PsR401* WT and mutant to be chosen on the basis of WP1
-The WP1 and 2 will likely identify additional functions that would enrich the WP3 targets
-A postdoctoral researcher and a PhD will be shared in between the WPs (see section II below for more information)

C2-Risk assessment and management

WPs are complementary with a **limited dependence** : if one WP fails it would have a limited impact on the other WPs.

WP1 : We do not anticipate major technical limitations in this WP as the genomic resources were previously generated (Getzke et al., 2023). The WP1 however represents heavy lab work, especially liquid handling and growth monitoring. The use of a liquid handling robot and/or multiplate reader is anticipated to speed up the process.

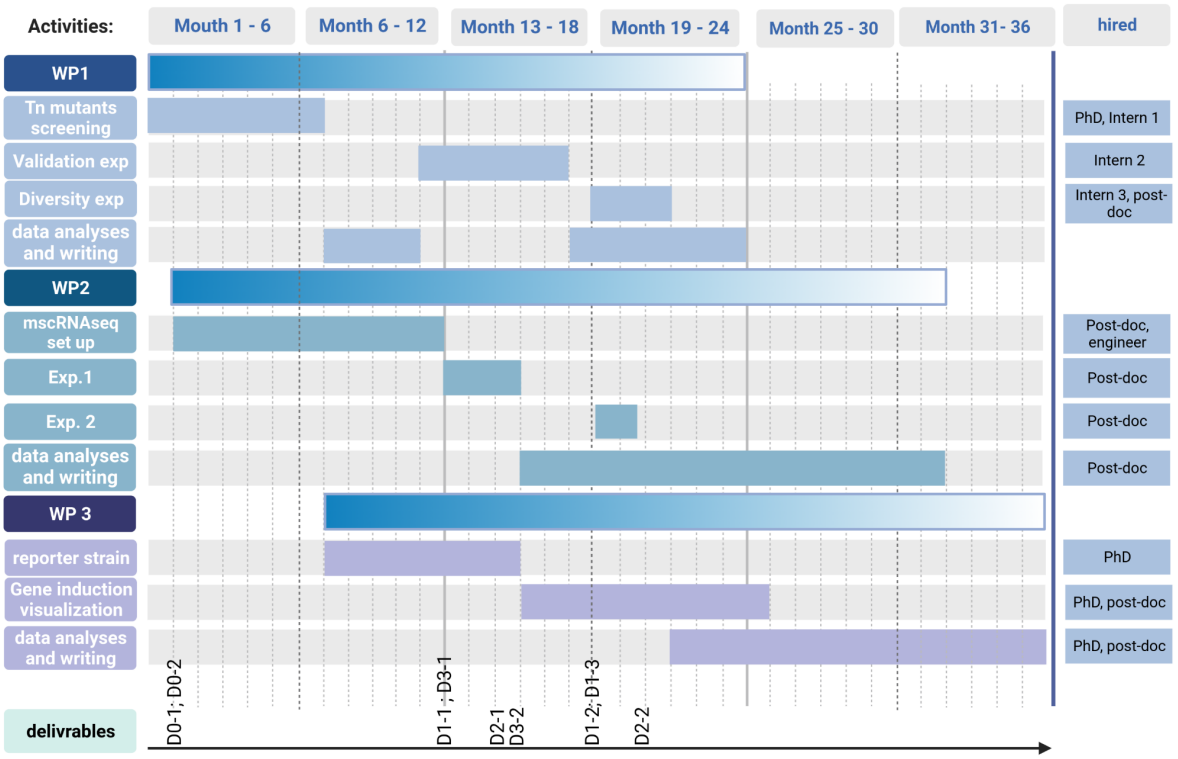
WP2 : The WP2 will develop on the basis of a validated protocol of bacterial single cell isolation and lysis compatible with molecular steps as a backbone. The development of a mscRNAseq protocol derived from the VASA seq strategy seems realistic. If we fail in this, 3 other published possible mscRNA-seq strategies would be possible to implement (Blattman et al., 2020; Imadahl et al., 2020; Kushina et al., 2021).

WP3 : Other functions than those targeted in WP3 will also be identified and incorporated via WP1 and WP2. Establishment of single-reporter strains (D3-1 below) can be used independently if difficulties occur during the production of the triple colour-reporter strain. Strain *PsR401* is genetically tractable and transformation is a routine procedure in the laboratory (Getzke et al. 2023).

C3- Deliverables

D0-1 Data management plan (Month 1)
D0-2 DivIDE Webpage (Month 1)
D1-1 List of candidate isolates (Month 12)
D1-2 Validation of candidate functions (Month 18)
D1-3 Candidate populations for diversity experiment (Month 18)
D2-1 mscRNAseq wetlab workflow validations (Month 14)
D2-2 mscRNAseq data analysis workflow (Month 20)
D3-1 single-reporter strains of PsR401 (Month 12)
D3-2 triple-reporter strain of PsR401 (Month 15)

C4- Gantt diagram



d. Positionnement du projet par rapport aux enjeux de recherche de l'axe scientifique choisi/Positioning of the project in relation to the research challenges of the chosen scientific axis

The project DivIDE fits perfectly within CE20 '*Axe A.3 : Biologie des animaux, des organismes photosynthétiques et des microorganismes*' which explicitly aims at fundamental research. The project will develop a better understanding of microorganisms in their interactions at the population level and interactions with a host plant and thus fits within the call for applications to study '[...] *des organismes photosynthétiques, modèles inclus, les organismes associés (microorganismes, microbiotes, ravageurs, pathogènes, auxiliaires...) et les interactions entre ces organismes' [...].*

Furthermore, within CE20-A3 the call for applications indicates that all the levels of regulation [...] *'(génomique, transcriptomique, épigénétique, traductionnelle, métabolique, physiologique, développemental)'*[...] are eligible. DivIDE will assess the genomic (WP1,2), transcriptomic (WP2), translational (WP3) and physiological (WP3) levels. The project fits within LS08-12 Microbial ecology and evolution, LS08-10 Ecology and evolution of species interactions, LS08-06 Evolutionary ecology, and LS02-07 Transcriptomics.

II. Organisation et réalisation du projet/Organization and realisation of the project

a. Coordinateur ou coordinatrice scientifique et son consortium / son équipe

Scientific coordinators in France and Germany:

Philippe Vandenkoornhuyse (Professor, Université de Rennes, UMR 6553 ECOBIO, WP2 leader) has mainly contributed to a better understanding of the plant microbiota. He recently developed new ideas and perspectives around the concept of holobiont and dysbiosis. He has also developed new knowledge on microorganism specialisation to explain the complexity of microbial communities. He is the scientific manager of the EcogenO platform (AnaEE research infrastructure). He has published 74 articles (>8000 citations), with an average journal Impact Factor of 9.98. During the last 10 years, he has supervised 5 post-doctoral researchers, 10 doctoral students and 16 Master's students. Over the past 5 years, P. Vandenkoornhuyse has coordinated 10 national and international projects (Europe, North America, China). He is currently a member of expert committees at HCERES, ANR, DFG (D), NWO (NL), FWO (B), NSF (USA), member of editorial boards or editorial committees such as, among others, ISME J.

Stephane Hacquard (group leader, Max Planck Institute, Cologne, WP3 leader) is an internationally-recognized, yet junior researcher, that has significantly contributed to advance the plant microbiota research field. Among the most influential discoveries, one can highlight the fact that 1) plants from diverse European habitats associate with the same small group of highly abundant microorganisms 2) microbe-microbe interactions in the microbiota promote host health or 3) bi-directional signalling along the microbiota-root-shoot axis is key to promote plant health. His research is ranked among the top 1% highly-cited work across multiple fields (https://clarivate.com/highly-cited-researchers/). Stéphane Hacquard has published > 50 articles (>7000 citations) and has an h-index of 29 (https://scholar.google.fr/citations?view_op=list_works&hl=fr&user=OnYeCdgAAAAJ). During the last five years, he has supervised 4 postdoctoral researchers, 7 PhD students, and several Master students. He obtained several prestigious grants, including an ERC starting grant in 2017 (MICRORULES), followed by an ERC consolidator grant in 2022 (MICROBIOSIS). He is associate editor for the journals "Plant, Cell & Environment", and "The New Phytologist".

Nathan Vannier (UMR IGEPP, WP1 leader)

Nathan Vannier is a junior researcher at the National Research Institute for Agriculture, Food and the Environment (INRAE). Expert in *in vitro* studies of plant-microbes and microbe-microbe interactions, and in the analysis of transcriptomics data, he contributed to the understanding of microbial functions involved in plant health and microbiota homeostasis by publishing 12 high impact articles (average IF 8,77). In addition, the IGEPP has recently acquired innovant culturomics equipment (Omnilog, automated liquid handling robot, Claryostar+) that will be a major asset to the project to permit high-throughput microbial interactions assays.

The other members of the DivIDE consortium :

UAR 3343 OSUR (Sophie Michon-Coudouel CNRS, Romain Causse-Védrines CNRS, Antoine Peigné Université de Rennes, engineers at EcogenO Platform)

Sophie Michon Coudouel is a CNRS engineer in molecular biology, expert in the production of sequencing libraries for Illumina and MGI technologies and has carried out innovative developments for mass sequencing (6 articles over the past 5 years).

Romain Causse-Vedrine is a CNRS engineer in molecular biology, high-throughput qPCR expert, who has developed robotization of protocols. He is also an expert in long-read sequencing library construction.

Antoine Peigné is responsible for the CellenOne instrument and participates in associated developments, especially microbial single cell whole genome sequencing (mscWGS).

S Michon Coudouel, R Causse-Védrines and A Peigné are involved in the development of the preparation of library construction in nano-volumes (miniaturisation).

All equipment required for the mscRNAseq are available at the EcogenO platform (Research Infrastructure ANAEE).

DivIDE to develop a collaborative research :

After a successful interaction between our labs with a shared PhD (Vitor Mataigne, PhD defended in dec 2021, Mataigne et al. 2021, Mataigne et al. 2022) new ideas with the need of collaborative work have been raised. The seminal work of Victor Mataigne suggests that metabolic interdependencies between bacteria is common and that community assembly rules involve co-metabolism among strains. From this former work we came to the conclusion that division of labour within microbiota also likely occurs at the intra-population level. To address hypotheses related to this idea, specific instruments and know how are required (1) regarding bacterial single cell -Omics at platform EcogenO leaded by P Vandenkoornhuyse (Univ Rennes, member of the Research infrastructure ANAEE-Fr and ANAEE-ERIC, UAR 3343 OSUR) and (2) culture collection, phenotyping and specific associated knowledges developed by S Hacquard and his group at the Max Planck Institute of Cologne). Nathan Vannier, a former Post-doc fellow within S Hacquard group now full researcher at INRAE, has developed important key skills for the success of the proposal especially regarding transcriptomic analyses and experimental work.

Tableau d'implication du coordinateur ou coordinatrice et des responsables scientifiques des partenaires dans d'autres projets en cours

Nom du ou de la participant.e au projet	Personne. mois	Intitulé de l'appel à projets, agence de financement, montant attribué	Titre du projet	Nom du coordinateur ou coordinatrice du projet	Date début - Date fin
Philippe Vandenkoornhuyse	12	ANR LABCOM (364,000€)	MICRObial Single Cell AnaLysEs Laboratory	Philippe Vandenkoornhuyse	2021-2025
Philippe Vandenkoornhuyse	7	PIA PPR Cultiver et Protéger autrement (3,000,000€ dont 407,000€ pour le laboratoire)	Deciphering plant-microbiota interactions to enhance crop defenses to pests – DEEP IMPACT	Christophe Mougel	2021-2025

AAPG2023	DivIDE		PRCI
Coordonné par :	Philippe VANDENKOORNHUYSE	36 months	Aide totale demandée ANR
CE20-A3 Biologie des animaux, des organismes photosynthétiques et des microorganismes LS08-12 Microbial ecology and evolution			

Stéphane Hacquard	5,4	DFG Grant SPP Decrypt (175,000€)	Bacterial genetic determinants preventing fungal dysbiosis in roots	Stéphane Hacquard	2022-2025
Stéphane Hacquard	36	ERC-CoG-2022 MICROBIOSIS (2,000,000€)	The microbiota-root-shoot axis in plant health and disease	Stéphane Hacquard	2023-2029
Nathan Vannier	NA	NA	NA	NA	NA

DivIDE to share :
Beside the complementarities, the collaborative research will also be developed by sharing the post doctoral researcher and PhD hired. In more detail, the PhD hired in Germany will also work at UMR IGEPP, a partner within DivIDE. The Post-doctoral researcher, based in Rennes, will also go to Germany and will be involved in WP3. The post-doctoral researcher will also share his/her working time at UMR IGEPP to boost the work to be completed within WP1.

b. Moyens mis en œuvre et demandés pour atteindre les objectifs

Partner 1 : ECOBIO

Frais de personnel / Personnel costs

Post-doctoral researcher to hire for 32 months : amount requested 145,000 €

Mainly involved in WP2, (i.e. estimated duration 18-20 months) but who will also bridge WP1 and WP3 (i.e. shared postdoc who will have to go in Germany 12-14 months) (see above for more details)

Coûts des instruments et du matériel / Costs of instruments and materials

Amount requested : 40,155 € (WP2)

- library construction to produce 2 x 1000 mscRNAseq including QCs, RNA fragmentase, Poly A tailing, ligases, end-repair, Phi29 DNA polymerase, microtubes, tips, gloves : 15,840 €

-library construction to produce 1000 mscWGS including QCs, RepliG & QiaseqFX kits : 10,660 €

-purchase of 500 sets of polyT-UMI-single cell barecode-sequencing adaptor : 5,855 €

-consumables for tests, quality checks, molecular grade basic reagents or solutions : 8,300 €

Coûts des bâtiments et des terrains / Costs of buildings and land NA

Coûts du recours aux prestations de service (et droits de propriété intellectuelle) / Costs of using services (and intellectual property rights)

Amount requested : 29,845 € (WP2) including 3 runs of Novaseq PE150

Frais généraux non-forfaitisés / General fees (meetings, conferences…)

Amount requested : 10,000 € of which, 3,000 € dedicated to participation at international conferences (2 events for 2), and 7,000 € for travels and stays in Germany.

Regarding the periods for the post-doctoral researcher of stays in Germany, it is planned to hire a studio at the Max Planck Institute (~500€/month). Regarding the publication fees, payments will be charged to the Max Planck Institute.

AAPG2023	DivIDE		PRCI
Coordonné par :	Philippe VANDENKOORNHUYSE	36 months	Aide totale demandée ANR
CE20-A3 Biologie des animaux, des organismes photosynthétiques et des microorganismes LS08-12 Microbial ecology and evolution			

Partner 2 : OSUR

Frais de personnel / Personnel costs

Molecular biology engineer to hire for 6 months : amount requested 20,000 €

Coûts des instruments et du matériel / Costs of instruments and materials

Molecular biology grade reagents, plastics for automatic dispenser, gloves etc. : **5000 €**

Coûts des bâtiments et des terrains / Costs of buildings and land NA

Costs of using services (and intellectual property rights) NA

Frais généraux non-forfaitisés NA

Partner 3 : IGEPP

Frais de personnel

Amount requested : 8,457€ (WP1)

- 2 MSc students for 6 months each : amount requested 6759**€** Involved in WP1, the first MSc student will carry-out primary screenings of mutants growth and the first screening of wild-type/mutants interaction. The shared post-doc will be involved in the supervision of the first MSc. The second MSc student will carry-out validation experiments and population diversity experiments. The shared PhD will be involved in these validation experiments and the diversity experiments.

- 1 Bsc student for 3 months : amount requested 1698**€**

The Bsc student will generate independent knock-out mutants for validation of candidate division of labour functions.

Coûts des instruments et du matériel

Amount requested : 11,390€ (WP1)

- Consumables for 15,000 *in vitro* growth assays (plastics for dispensers, gloves, microbial culture medias): estimated costs 4600€ ; molecular grade basic reagents or solutions for estimated 300 DNA extractions and PCR reactions : 300*10€ = 3000€

- Multi-channel microlitre electronic pipette (10µL-100µL) : 739€

- Multi-channel microlitre electronic pipette (100µL-1000µL) : 1,151€

- Gibson assembly cloning kits for independent gene validation : 19€ per unit = 19*100 = 1900€

Coûts des bâtiments et des terrains NA

Coûts du recours aux prestations de service (et droits de propriété intellectuelle)

Amount requested : 3348€

- 2 years Geneious prime licence for genomic analysis and microbial genomes engineering : amount requested 1050€

- 96 reactions Mix2Seq Kits for sanger sequencing to identify transposon insertion sites and knock-out mutants generations quality checks : 6*383€ = 2298€

Frais généraux non-forfaitisés

Amount requested : 2,000 € dedicated to participation at international conferences

Partner 4 : Max Planck Institute

Frais de personnel

Doctoral researcher to be hired for 36 months : amount requested: 144,495 €.

The PhD student will be involved in WP1 and WP3. Her/his salary will be 65 % of E13 TVöD-Bund (48 165 €/year), which corresponds to 144 495 € for three years.

Coûts des instruments et du matériel

Amount requested : 31,300€

- Basic laboratory consumables i.e. microbial growth media (1,500€), circle and square petri dishes (3,000€), gloves (300€), gellan gum and alginate for transparent soil production (400€).
- Molecular Biology : costs for primer synthesis (200 primers, average of 3 euros per primer: 600€) and plasmid orders (400€) ; Costs for DNA and RNA isolation kits (plants and or bacterial DNA, FastDNA SpinKit, 200 reactions: 800€, RNAeasy minikit, 50 reactions: 500€). Superscript reverse transcriptase kit (2,000 units: 150€ and SYBR Green qPCR Master mix (350€) for promoter reporter line validation with native gene expression ; Costs for high fidelity Taq polymerase for PCR amplification (Fusion polymerase, 3000€) classical Taq DNA polymerase (500€), PCR digestion enzymes (antarctic phosphatase, exonuclease: 1,000€), PCR purification kits (PCR gel clean-up: 1,000€, DynaMag magnet + beads: 3,000€) ; ligation and restriction enzymes (500€), competent E. coli cells (600€) as well as antibiotics (100€) ; Gibson assembly cloning kit for 200 reactions (19€ per reaction: 3,800€) ; 2 MiSeq cartridges to analyse SynCom community composition in response to iron levels and PsR401 presence (3600€) ; Mix2Seq Kits for sanger sequencing to validate PCR products, insertion sites, and plasmid construction: 200 reactions: 4 600€ ; 3 years Geneious prime licence for primer design and genomic-based analyses: amount requested 1 600€

Coûts des bâtiments et des terrains NA

Coûts du recours aux prestations de service (et droits de propriété intellectuelle) NA

Frais généraux non-forfaitisés

Amount requested : 8,250 € of which, 6,000 € will be dedicated to travel (3 international conferences during the 3-year project) and travel/accomodation for a 6-month stay in Rennes and 2,250 € for publication costs. Regarding the stay in France, a studio will be rented at Cité Internationale Paul Ricoeur (~500€/month). The articles will be published open access in international journals that are part of the open access project DEAL. Therefore, most of the publication costs will be covered by the Max Planck Society via this DEAL program. We therefore only request 2,250 € for publication costs.

AAPG2023	DivIDE		PRCI
Coordonné par :	Philippe VANDENKOORNHUYSE	36 months	324,095 €
A3 Biologie des animaux, des organismes photosynthétiques et des microorganismes LS08-12 Microbial ecology and evolution			

Moyens demandés par grand poste de dépense et par partenaire / Resources requested by major item of expenditure and by partner

	Partenaire *ECOBIO*	**Partenaire** *OSUR*	**Partenaire** *IGEPP*	**Partenaire** *MAX PLANCK INSTITUTE*
Frais de personnels, y compris éventuelle décharge d'enseignement dans le cadre JCJC	145,000	20,000	8,457	144,495
Coûts des instruments et matériels	40,155		11,390	31,300
Coûts des bâtiments et des terrains				NA
Prestation de service et droits de propriété intellectuelle	29,845	5,000	3,348	NA
Frais généraux non forfaitisés	10,000		2,000	8,250
Préciput pour un partenaire public / Frais d'environnement pour un partenaire privé **	30,375	3,375	3,401.33	overheads (22% for DFG) 40,489
Sous-total	**255,375**	**28,375**	**28,596.33**	**224,534.9**
Aide demandée	**(ANR = 314,095.43 € + DFG = 224,534.9 €) = 538,640 €**			

III. Impact et retombées du projet

DivIDE is a fundamental research project which aims to better understand the complexity of microbial communities and microbiota. DivIDE has the ambition to define a novel research line in the plant microbiota research field and to identify multiple mechanisms that promote functional diversity in a genetically identical bacterial population. The impact of the DivIDE research is expected to be broad : by its novelty it can impact different fields of research including primarily plant science and microbiology, but also evolutionary biology, ecology, evolutionary ecology, microbiota research, biotechnologies (i.e. mscRNAseq workflow), bioinformatics.

-The research within DivIDE is expected to lead to 3-5 publications published in the very best scientific journals. These publications will be Open Access.

-Within the lifetime of the project, we will develop different communication actions through the DivIDE website, including digests of the research for the media and for non-scientific readers.

-We will interact with a Biotech company (Cellenion Lyon).

-We will develop bridges between Rennes (UMRs ECOBIO and IGEPP; OSUR) and the Max Planck Institute of Cologne in the field of plant-microbe interaction (i.e. bilateral event), and also possibly by the co-organisation of a Jacques Monod conference (application in 2024 or 2025).

-Finally, a remote and cross-border teaching unit '*Frontiers in ecological- evolutionary- and environmental- genomics*' for Master students and PhDs, in which the DivIDE consortium will take part, will start in September 2023 (starting of the TU from sept 2023, Resp P Vandenkoornhuyse & L Bittner).

IV. Bibliographie

Adkins-Jablonsky SJ, Clark CM, Papoulis SE, Kuhla MD, Morris JJ (2021) Market forces determine the distribution of a leaky function in a simple microbial community. Proc Natl Acad Sci 118 : e2109813118. doi 10.1073/pnas.2109813118

Blattman SB, Jiang W, Oikonomou P, Tavazoie S (2020) Prokaryotic single-cell RNA sequencing by in situ combinatorial indexing. Nat Microb 5:1192–1201. doi 10.1038/s41564-020-0729-6

Brameyer S, Schumacher K, Kuppermann S, Jung K (2022) Division of labor and collective functionality in Escherichia coli under acid stress. Commun Biol 5:327. doi 10.1038/s42003-022-03281-4

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR (2013) STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29:15–21. doi 10.1093/bioinformatics/bts635

Estrela E, Morris JJ, Kerr B (2016) Private benefits and metabolic conflicts shape the emergence of microbial interdependencies. Environ Microbiol 18:1415–1427.

Getzke F, Hassani MA, Crüsemann M, Malisic M, Zhang P, Ishigaki Y, Böhringer N, Jiménez Fernández A, Wang L, Ordon J, Ma K-W, Thiergart T, Harbort CJ, Wesseler H, Miyauchi S, Garrido-Oter R, Shirasu K, Schäberle TF, Hacquard S, Schulze-Lefert P (2023) Co-functioning of bacterial exometabolites drives root microbiota establishment. Proc Natl Acad Sci, in press

Gu S, Wei Z, Shao Z, Friman V-P, Cao K, Yang T, Kramer J, Wang X, Li M, Mei X, Xu Y, Shen Q, Kümmerli R, Jousset A (2020) Nat Microb 5 : 1002–1010. doi 10.1038/s41564-020-0719-8

Han J, Xia A, Huang Y, Ni L, Chen W, Jin Z, Yang S, Jin F (2019) Simultaneous Visualization of Multiple Gene Expression in Single Cells Using an Engineered Multicolor Reporter Toolbox and Approach of Spectral Crosstalk Correction. ACS Synth Biol. 15:2536-2546. doi: 10.1021/acssynbio.9b00223

Harbort CJ, Hashimoto M, Inoue H, ..., Sattely ES, Garrido-Oter R, Schulze-Lefert P (2020) Cell Host Microb 28:825–837. doi: 10.1016/j.chom.2020.09.006

Imdahl F, Vafadarnejad E, Homberger C, Vogel J (2020) Single-cell RNA-sequencing reports growth-condition-specific global transcriptomes of individual bacteria. Nat Microb 5:1202–1206. doi 10.1038/s41564-020-0774-1

Knights HE, Jorrin B, Haskettand TL, Poole PS (2021) Deciphering bacterial mechanisms of root colonization. Env Microb Rep 13(4), 428–444. doi: 10.1111/1758-2229.12934

Kuchina A, Brettner LM, Paleologu L, Roco CM, Rosenberg AB, Carignano A, Kibler R, Hirano M, DePaolo RW, Seelig G (2021) Microbial single-cell RNA sequencing by split-pool barcoding. Science 371, eaba5257. doi 10.1126/science.aba5257

Lim CK, Hassan K A, Tetu SG, Loper JE, Paulsen IT (2012). The Effect of Iron Limitation on the Transcriptome and Proteome of Pseudomonas fluorescens Pf-5. PloS ONE, 7:e39139. doi: 10.1371/journal.pone.0039139

Lopez JG, Wingreen NS (2022) Noisy metabolism can promote microbial cross-feeding. eLife 11:e70694 doi 10.7554/eLife.70694. doi: 10.7554/eLife.70694

Luecken MD, Theis FJ (2019) Current best practices in single-cellRNA-seq analysis: A tutorial. Mol Syst Biol 15:e8746. doi 10.15252/msb.20188746

Ma L, Shi Y, Siemianowski O, Cademartiri L (2019) Hydrogel-based transparent soils for root phenotyping in vivo. Proc Natl Acad Sci USA 116:11063-11068. doi 10.1073/pnas.1820334116

Mataigne V, Vannier N, Vandenkoornhuyse P, Hacquard S (2021) Microbial Systems Ecology to Understand Cross-Feeding in Microbiomes Front. Microb 12:780469. doi: 10.3389/fmicb.2021.780469

Mataigne V, Vannier N, Vandenkoornhuyse P, Hacquard S (2022) Multi-genome metabolic modelling predicts functional inter-dependencies in the Arabidopsis root microbiota. Microbiome, 10:217. doi: 10.1186/s40168-022-01383-z

Mo Y-Y, Gross D C (1991) Expression in vitro and during plant pathogenesis of the syrB gene required for syringomycin production by Pseudomonas syringae pv. syringae. Mol Plant-Microbe Interact. 4:28–36. doi 10.1094/MPMI-4-028

Palma M, Worgall S, Quadri LEN (2003). Transcriptome analysis of the Pseudomonas aeruginosa response to iron. Arch Microb 180:374–379. 10.1128/JB.186.1.248-252.2004

Papalexi E, Satija R (2018) Single-cell RNA sequencing to explore immune cell heterogeneity. Nature Reviews Immunology, 18, 35–45. doi 10.1038/nri.2017.76

Rosenthal AZ, Qi Y, Hormoz S, Park J, Hsin-Jung Li S, Elowitz MB (2018) Metabolic interactions between dynamic bacterial subpopulations eLife 7:e33099. doi 10.7554/eLife.33099

Salmen F, De Jonghe J, Kaminski TS, Alemany A, Parada GE, Verity-Legg J, Yanagida A, Kohler TN, Battich N, van den Brekel F, Ellermann AL, Martinez Arias A, Nichols J, Hemberg M, Hollfelder F, van Oudenaarden A (2022) High-throughput total RNA sequencing in single cells using VASA-seq. Nat Biotech 40:1780–1793 doi 10.1038/s41587-022-01361-8

Stuart T, Butler A, Hoffman P, Hafemeister C, Papalexi E, Mauck 3rd WM, Hao Y, Stoeckius M, Smibert P, Satija R (2019), Comprehensive integration of single-cell data. Cell 177:1888–1902.e21 doi: 10.1016/j.cell.2019.05.031

Wolf FA, Angerer P, Theis FJ (2018) SCANPY: Large-scale single-cell gene expression data analysis. Genome Biol. 19:15. doi 10.1186/s13059-017-1382-0

Zelezniak A, Andrejev S, Ponomarova O, Mende DR, Bork P, Patil KR (2015) Metabolic dependencies drive species co-occurrence in diverse microbial communities. Proc Natl Acad Sci U S A 112:6449–54. doi 10.1073/pnas.1522642113.