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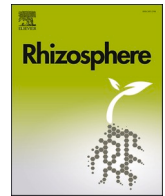
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When roots talk, bacteria respond: Comparative transcriptomics of three plant growth-promoting rhizobacteria growing on root exudates reveals plant-specific responses

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ABSTRACT

In the rhizosphere, plant roots release rhizodeposits, including a complex mixture of molecules known as root exudates. These compounds mediate the recruitment of the rhizosphere microbiota, which plays a key role in plant growth and resistance to biotic and abiotic stresses. Deciphering the molecular dialogue between roots and their microbiota could promote the establishment of bioinoculants, such as plant growth-promoting rhizobacteria (PGPR), in the rhizosphere. Here, we investigated the genetic responses of three PGPR strains (*Bacillus subtilis* ATCC 6633, *Pseudomonas fluorescens* ATCC 17400 and *Azospirillum brasilense* Sp245) to root exudates from rapeseed (*Brassica napus*), pea (*Pisum sativum*) and ryegrass (*Lolium perenne*). Transcriptomic analyses during PGPR growth on root exudates revealed that *B. subtilis* ATCC 6633 and *A. brasilense* Sp245 regulated the expression of hundreds of genes, while *P. fluorescens* ATCC 17400 appeared to already express most of the genes required for this response. Moreover, certain bacterial genes, mainly involved in metabolism, were regulated by all three exudates through a core transcriptome, while other genes were specifically modulated by each plant. Notably, rapeseed exudates stimulated amino acid metabolism, whereas pea exudates repressed motility and activated defense mechanisms. Finally, we deduced the amino acids and sugars present in root exudates and metabolized by PGPR. Together, our results highlight root exudate specificity in the recruitment of PGPR and could contribute to their establishment as bioinoculants for rhizosphere engineering.

1. Introduction

Climate change, combined with intensive farming systems, industrial fertilizer inputs and pesticide use, greatly affects agroecosystems and, therefore, global crop yields (George et al., 2024; Oburger et al., 2022). To meet the growing demand for food from the growing population, sustainable agriculture has emerged to address these challenges while ensuring human and environmental health (Boros et al., 2025). Sustainable agricultural practices include crop diversification and rotation, intercropping, and organic farming (Boros et al., 2025). Moreover, substances or microorganisms that improve plant nutrient use efficiency and/or abiotic stress tolerance (i.e. biostimulants) and protect plants from biotic stresses (i.e. biocontrol products) can enhance crop production (Galli et al., 2024; Zulfiqar et al., 2024). In particular, bioinoculants are formulations of microorganisms that, when applied to plants or their rhizosphere, can act as biofertilizers or biopesticides (Maitra et al., 2022).

Plant growth-promoting rhizobacteria (PGPR) are often used as bioinoculants because of their wide range of beneficial effects (Basu et al., 2021; Cunha et al., 2024). Indeed, PGPR can promote plant growth by enhancing nutrient acquisition (e.g. nitrogen, phosphorus, iron), modulating phytohormone homeostasis (e.g. auxins, cytokinins), and improving plant tolerance to abiotic and biotic stresses (Altaf et al., 2026; Ehinmitan et al., 2024; Hasan et al., 2021; Shah et al., 2021). In particular, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Azospirillum brasilense* are well-described PGPR species sold as biofertilizer products (Basu et al., 2021). *B. subtilis* can enhance nutrient availability, modulate phytohormones, form protective biofilms, stimulate plant induced systemic resistance, and synthesize antimicrobial compounds (Blake et al., 2021). *P. fluorescens* can solubilize phosphate, synthesize phytohormones, and compete with phytopathogens through antimicrobial and siderophore production (David et al., 2018). *A. brasilense* can fix nitrogen, modulate phytohormones, induce plant systemic resistance, and improve drought or salinity tolerance by increasing osmolyte

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concentrations in plant cells (Fukami et al., 2018; Zannis-Peyrot et al., 2025).

To exert their beneficial effects, PGPR must reach the rhizosphere, i.e. the narrow zone of soil surrounding and influenced by plant roots (Solomon et al., 2024; Venturi and Keel, 2016). Roots release rhizodeposits, including the root extracellular trap (i.e. the network of mucilage and root-associated cap-derived cells), root debris, extracellular vesicles and root exudates, which together select soil microorganisms and shape the rhizosphere microbiota (Ambrosone et al., 2023; Driouich et al., 2021; Gaudry et al., 2025; Philippot et al., 2013; Tian et al., 2020). In particular, root exudates are a complex and dynamic molecular mixture including primary metabolites (e.g. carbohydrates, amino acids, organic acids) and secondary metabolites (e.g. flavonoids, glucosinolates), which are challenging to collect and characterize (Afridi et al., 2024; McLaughlin et al., 2023; Oburger and Jones, 2018; Vives-Peris et al., 2020).

Root exudates have been shown to recruit beneficial microorganisms such as PGPR by attracting and feeding them, or even inducing their biofilm formation (Chen and Liu, 2024; Dhungana et al., 2023; Sasse et al., 2018; Shafi and Shahid, 2025). However, root exudates may also contain antimicrobial or repulsive compounds that contribute to plant defense against phytopathogens (Lamichhane et al., 2024). The establishment of plant-specific rhizosphere microbiota thus raises the question of the specificity of root exudates in the selection of microorganisms. Previously, we studied and compared the physiological responses of three PGPR strains (*B. subtilis* ATCC 6633, *P. fluorescens* ATCC 17400, and *A. brasilense* Sp245) to root exudates from three plant species of agricultural interest: rapeseed (*Brassica napus*), pea (*Pisum sativum*), and ryegrass (*Lolium perenne*). Thus, different chemotactic responses and growth parameters were identified between plant-bacteria pairs and evaluated by a “love match” score (Fourné et al., 2024).

Here, we sought to identify the genetic responses of these PGPR strains to the same root exudates in order to better understand the specific bacterial behaviors induced by exudates. Therefore, a genomic analysis was conducted first to compare the potential functions of *B. subtilis* ATCC 6633, *P. fluorescens* ATCC 17400, and *A. brasilense* Sp245, including their plant growth-promoting traits. Secondly, a transcriptomic analysis was performed to identify bacterial gene expression during growth on root exudates from rapeseed, pea and ryegrass. This study should help determine which mechanisms are involved in the specific selection of rhizosphere microbiota populations and to identify root exudate compounds that regulate bacterial metabolism.

2. Materials and methods

2.1. Plant material, growth conditions and root exudate collection

Rapeseed (*Brassica napus* var. Aviso), pea (*Pisum sativum* var. Astro-naute), and ryegrass (*Lolium perenne* var. Delika) were cultivated and their root exudates collected as described by Fourné et al. (2024). Briefly, plants were grown under hydroponic conditions for approximately five weeks, and their root exudates were collected by rinsing the roots for a few seconds and then immersing them in sterile ultrapure water for 1 h. Root exudates were centrifuged at 10,000 rpm for 15 min to collect the supernatant, which was then lyophilized and resuspended in sterile ultrapure water to concentrate. Sterile hydroponic solutions were used to minimize contamination, but it was not possible to maintain sterile conditions throughout the plant culture. Microbial development was controlled by plating exudates on an LB Petri dish. Carbon (C) and nitrogen (N) concentrations of root exudates were determined by Isotope Ratio Mass Spectrometry (IRMS) by the PLATIN[®] platform (University of Caen Normandie), which indicated a mean C concentration of 336 mg/L and a mean N concentration of 44 mg/L (Fourné et al., 2024). Several root exudate collection campaigns were conducted for each plant species to test the variability of exudate samples between

campaigns.

2.2. Bacterial strains and growth conditions

The bacterial strains *Bacillus subtilis* ATCC 6633, *Pseudomonas fluorescens* ATCC 17400, and *Azospirillum brasilense* Sp245, were recently reclassified as *Bacillus spizizenii* (Dunlap et al., 2020), *Pseudomonas proteolytica* (unpublished results), and *Azospirillum baldaniorum* (dos Santos Ferreira et al., 2020), respectively. Given the extensive literature, including recent publications, that refer to these strains as *B. subtilis*, *P. fluorescens*, and *A. brasilense*, and considering that their reported beneficial effects are consistent with those of these species, the three strains will be referred to as *B. subtilis* ATCC 6633, *P. fluorescens* ATCC 17400, and *A. brasilense* Sp245 in this article.

B. subtilis ATCC 6633 and *P. fluorescens* ATCC 17400 were grown in 500 µL M9 minimal medium (3 g/L KH₂PO₄, 0.5 g/L NaCl, 6.78 g/L Na₂HPO₄, 1 g/L NH₄Cl, 0.24 g/L MgSO₄, 14.7 mg/L CaCl₂, 3.6 g/L glucose), and *A. brasilense* Sp245 in 500 µL modified M9 medium (3 g/L KH₂PO₄, 0.5 g/L NaCl, 6.78 g/L Na₂HPO₄, 1 g/L NH₄Cl, 0.24 g/L MgSO₄, 14.7 mg/L CaCl₂, 5 mg/L biotin, 2.5 mg/L FeSO₄, 2.5 g/L malate) in horizontal hemolysis tubes for 24 to 32 h (bacterial density $N \approx 1.10^9$ CFU/mL) with stirring (120–140 rpm). *B. subtilis* and *P. fluorescens* were grown at 30 °C and *A. brasilense* at 25 °C. To study their growth on root exudates, 1 µL of this M9 preculture was used to inoculate a new tube containing 250 µL PBS 2X (16 g/L NaCl, 0.4 g/L KCl, 2.88 g/L Na₂HPO₄, 0.49 g/L KH₂PO₄, pH 7.4) supplemented with 250 µL of root exudates, which was incubated until the middle or late exponential phase ($N \approx 10^7$ CFU/mL). Bacterial strains were also cultivated in 500 µL M9 and 500 µL LB rich medium (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl) as controls ($N \approx 10^7$ CFU/mL). For each plant species, four biological replicates were performed with root exudate samples from four different collection campaigns. M9 and LB cultures were replicated eight and four times, respectively.

2.3. RNA extraction and sequencing

Each 500 µL bacterial culture was duplicated to obtain a 1 mL suspension, from which 10 µL were taken to verify bacterial density by enumeration on LB agar plates. Total RNA from the remaining bacterial suspension was extracted using the PureLink[™] RNA Mini Kit (Invitrogen) and sent to Novogene GmbH (Munich, Germany), which performed mRNA-seq (Illumina NovaSeq 6000). Sequences were provided in FASTQ format. At least four biological replicates were performed for each condition.

2.4. Genomic analyses

The genomes of *B. subtilis* ATCC 6633 (GenBank ID = GCA_006094475.1) and *A. brasilense* Sp245 (GenBank ID = GCA_003119195.2) were obtained from the NCBI database (<https://www.ncbi.nlm.nih.gov/>). For *P. fluorescens* ATCC 17400, its DNA was extracted using the NucleoSpin[®] Microbial DNA Kit (MACHEREY-NAGEL) and sent to Novogene GmbH (Munich, Germany), which performed whole-genome sequencing (PacBio SMRT), assembly, and annotation. The genome sequence is available on the NCBI database (GenBank ID = GCA_00708695.3).

Bacterial genome coding DNA sequences were analyzed using the eggNOG-mapper tool (<http://eggnog-mapper.embl.de/>) with default settings to assign each gene to one or more functional categories from the Clusters of Orthologous Genes (COG) database (<https://www.ncbi.nlm.nih.gov/research/cog/>).

The plant growth-promoting traits (PGPTs) were identified in each bacterial genome using the PLant-associated Bacteria web resource (PLaBAs, v.1.02) (Ashrafi et al., 2022; Patz et al., 2021). PLaBAs was chosen for its accessibility, its relevance to our study, and its analysis tool (PGPT-Pred) that, based on a large number of protein sequences,

predicts PGPTs from any bacterial genome. The protein sequences of *B. subtilis* ATCC 6633, *P. fluorescens* ATCC 17400, and *A. brasilense* Sp245 were obtained from the NCBI database, uploaded to the PGPT-Pred tool, and aligned with blastp + hmmer (strict mode). The output file associates proteins with one or more PGPTs described by six levels of precision, and was used to generate a heatmap with RStudio software (v4.4.3; R Core Team, 2023).

2.5. Transcriptomic analyses

FASTQ files from RNA-seq were analyzed using the tool suite BBtools 39.01 (Bushnell et al., 2017). First, low quality reads (Qscore <20, length <105 bp) were removed with BBduk. Paired-end reads were merged with BBmerge and aligned to the bacterial genome with STAR 2.7.10a (Dobin et al., 2013). Counting was done with Cufflinks 2.2.1 to obtain the number of Fragments Per Kilobase of transcript per Million mapped reads (FPKM) (Trapnell et al., 2010). At least four biological replicates were used for each condition. Differential gene expression analysis was conducted with DESeq2 1.42.0 on RStudio software (v4.3.1; R Core Team, 2023) using the fold change measure. Genes differentially expressed by bacteria on root exudates compared to M9 medium were identified when log₂(fold change) ≥2 or ≤ -2 and p-adjust ≤0.05. RStudio software was also used to create heatmaps, principal component analyses, and volcano plots. Venn diagrams were generated using an online tool (<https://bioinformatics.psb.ugent.be/webtools/Venn/>). Functional annotation of differentially expressed genes was based on the COG database, as described for genomic analyses. Additionally, the functions of some genes were investigated further using Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto, 2000).

2.6. Accession numbers

RNA sequences generated and used in this study have been deposited in the Sequence Read Archive database and are available with BioProject accession number PRJNA1390816.

3. Results

3.1. Genomic analysis of PGPR strains

The genomes of the plant growth-promoting rhizobacteria (PGPR) strains *Bacillus subtilis* ATCC 6633, *Pseudomonas fluorescens* ATCC 17400 and *Azospirillum brasilense* Sp245 were analyzed to identify and compare their genomic features, including functions related to plant growth-promotion and responses to root exudates.

3.1.1. General genome features

B. subtilis ATCC 6633 is a Gram-positive bacterium of the phylum Bacillota with a 4 Mb chromosome encoding 4,144 genes, including 3,891 protein-coding genes, which therefore has the smallest genome

among the three strains (Table 1). *P. fluorescens* ATCC 17400 is a Gram-negative bacterium of the phylum Pseudomonadota (Gammaproteobacteria) with a 6.3 Mb chromosome encoding 5,794 genes, including 4,839 protein-coding genes. *A. brasilense* Sp245 is a Gram-negative bacterium of the phylum Pseudomonadota (Alphaproteobacteria) with a 3 Mb chromosome, three chromids of 1.8 Mb (p02), 925 kb (p08) and 740 kb (p10), and three plasmids of 810 kb (p03), 183 kb (p04) and 196 kb (p09), totaling 7.7 Mb (Wisniewski-Dyé et al., 2011). This represents the largest genome of the three strains, encoding 7,004 genes which include 6,788 protein-coding genes. Comparison of the three PGPR genomes shows that *A. brasilense* Sp245 has the highest GC% (68.5%), *P. fluorescens* ATCC 17400 has an intermediate value (60.5%), while *B. subtilis* displays the lowest (44%) (Table 1). These differences correlate with genome size, as reported in the literature (Nishida, 2012).

3.1.2. Comparative functional genomics

The three genomes were analyzed using the Clusters of Orthologous Genes (COG) database, which classifies genes into 26 categories (A-Z). The results were expressed as the percentage of genes involved in each COG category, allowing comparison regardless of genome size (Fig. 1). Categories A, B, R, W, X, Y and Z, which represent less than 0.1% of the genes for all three strains, were excluded in this study. It should be noted that approximately 30% of the genes in all three studied strains have an unknown function, which corresponds to the average annotation of bacterial genomes (Tatusov et al., 2000).

The five most represented functional categories in the genomes of *P. fluorescens* ATCC 17400 and *A. brasilense* Sp245 are the same, i.e. amino acid transport and metabolism (E), signal transduction mechanisms (T), transcription (K), inorganic ion transport and metabolism (P), and energy production and conversion (C) (Fig. 1). Despite a smaller genome size, the functional distribution within the *B. subtilis* ATCC 6633 genome is rather similar to that of *P. fluorescens* ATCC 17400 and *A. brasilense* Sp245, as three of the top five categories are common to all three strains studied (E, K and P), while the other two correspond to carbohydrate transport and metabolism (G), and envelope biogenesis (M).

Comparing the percentages of genes involved in all functional categories, *B. subtilis* ATCC 6633 appears to have a higher proportion of genes associated with carbohydrate transport and metabolism, and translation than the other two strains. In contrast, *P. fluorescens* ATCC 17400 and *A. brasilense* Sp245 have more genes involved in energy production and conversion, cell motility, transport and metabolism of secondary metabolites, and signal transduction mechanisms than *B. subtilis* ATCC 6633. These differences likely reflect the fact that *B. subtilis* is a Gram-positive bacterium and therefore phylogenetically more distant from *P. fluorescens* and *A. brasilense*, which are both Gram-negative bacteria belonging to the same phylum Pseudomonadota.

Between the two Gram-negative bacteria, *P. fluorescens* ATCC 17400 has a higher proportion of genes related to nucleotide, lipid and inorganic ion transport and metabolism, as well as genes involved in transcription, cell motility and vesicular transport than *A. brasilense* Sp245.

Table 1
General genome features of the three bacteria.

Feature		<i>Bacillus subtilis</i> ATCC 6633	<i>Pseudomonas fluorescens</i> ATCC 17400	<i>Azospirillum brasilense</i> Sp245
Size (Mb)		4.0	6.3	7.7
GC content (%)		44.0	60.5	68.5
Total number of genes		4,144	5,794	7,004
Number of protein-coding genes		3,891	4,839	6,788
Number of non-coding RNA genes	Total	121	91	123
	rRNA (5S, 16S, 23S)	10, 10, 10	7, 6, 6	9, 10, 10
	tRNA	86	68	90
	Other RNA	5	4	4
Number of pseudogenes		132	864	93
GenBank ID		GCA_006094475.1	GCA_000708695.3	GCA_003119195.2

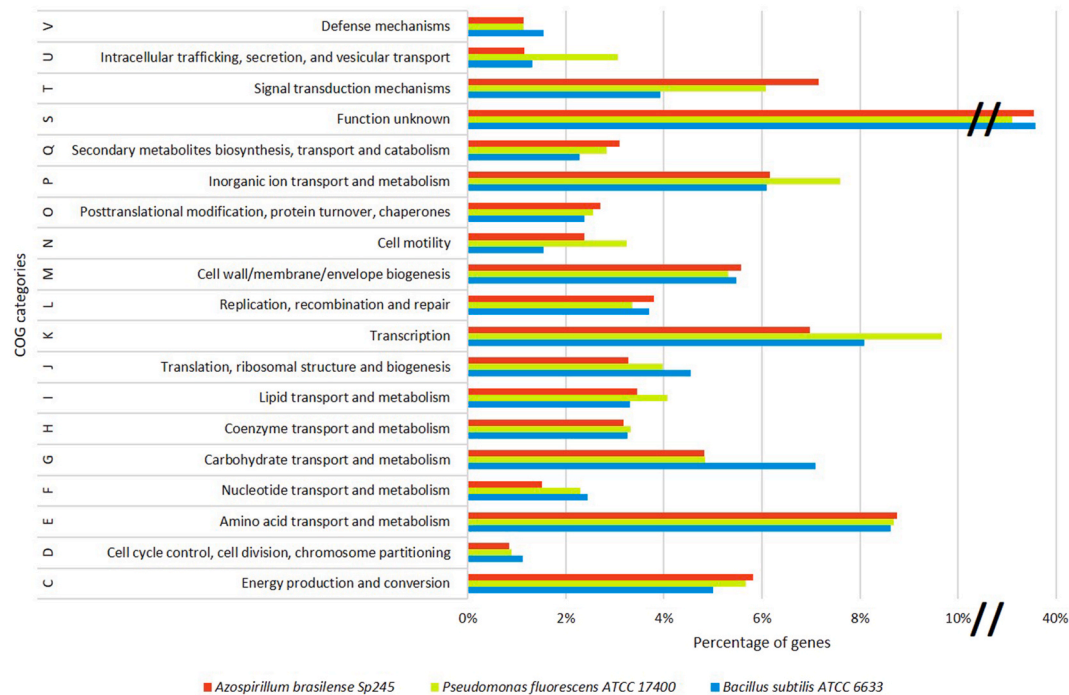


Fig. 1. Comparison of COG functional categories between the genomes of the three bacteria.

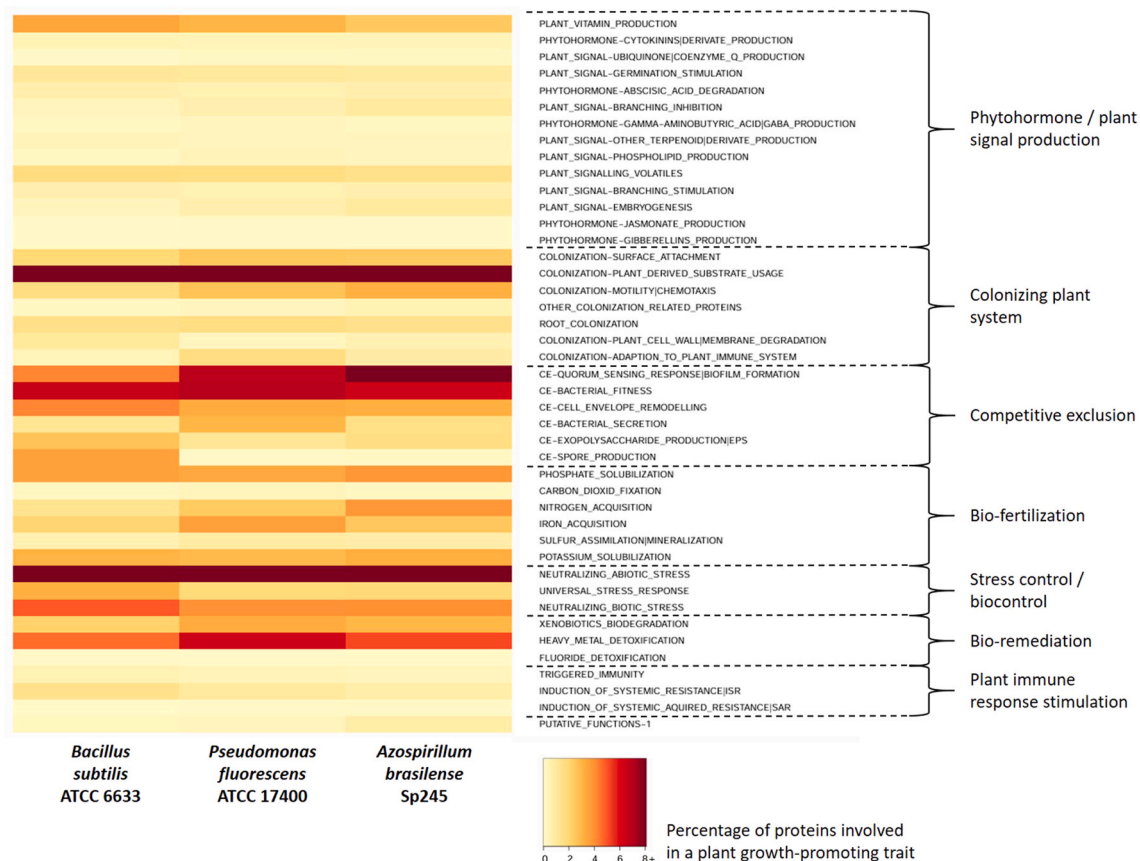


Fig. 2. Heatmap comparing the distribution of plant growth-promoting traits between the genomes of the three bacteria. The colored horizontal lines represent the percentage of predicted proteins involved in each function. Due to their high percentages, two functions fall outside the color range (dark red): the “colonization – plant-derived substrate usage” function represents 20.63%, 16.43%, and 17.88% of the predicted proteins of *Bacillus subtilis* ATCC 6633, *Pseudomonas fluorescens* ATCC 17400, and *Azospirillum brasilense* Sp245, respectively, while the “neutralizing abiotic stress” function represents 12.49%, 12.71%, and 11.80% of each strain's predicted proteins.

In contrast, *A. brasilense* Sp245 has more genes involved in signal transduction mechanisms than the other two strains. Taken together, these results show that the genomes of the three PGPR strains have similar proportions of functions potentially involved in root exudate responses. However, differences were also observed for some functional categories, highlighting the unique characteristics of each species.

3.1.3. Plant growth-promoting traits

To gain more information on bacterial functions, specifically on their plant growth-promoting traits (PGPTs), a genomic protein annotation was conducted using the PGPT prediction tool of PLaBase. Whereas the three PGPR strains *B. subtilis* ATCC 6633, *P. fluorescens* ATCC 17400 and *A. brasilense* Sp245 have already been shown to exhibit certain PGPTs (Bartolini et al., 2017; Cohen et al., 2015; Planchon et al., 2021; Yadav et al., 2021; Ye et al., 2014; Yu et al., 2021; Zou et al., 2024), this analysis allowed to identify and compare their potential PGPTs from genomic protein sequences.

Interestingly, 2,432 proteins of *B. subtilis* ATCC 6633 are involved in at least one PGPT, representing 62.5% of its total proteome. In *P. fluorescens* ATCC 17400, 2,810 proteins are associated with PGPTs, accounting for 58.1% of its proteome. *A. brasilense* Sp245 has 3,749 proteins involved in one or more PGPTs, representing 55.8% of its proteome. Thus, more than half of the predicted proteins in the three bacterial strains are involved in direct or indirect plant growth-promoting functions.

The PGPT distribution is relatively similar among the three bacteria (Fig. 2). Specifically, the profiles of *P. fluorescens* ATCC 17400 and *A. brasilense* Sp245 are more similar to each other than to *B. subtilis* ATCC 6633, reflecting their phylogenetic proximity. Visible differences include a higher percentage of proteins involved in plant-derived substrate usage, exopolysaccharide production and spore production in *B. subtilis* ATCC 6633. In contrast, *P. fluorescens* ATCC 17400 and

A. brasilense Sp245 have a higher percentage of proteins involved in motility and chemotaxis, as well as in *quorum sensing* response for bio-film formation. Furthermore, *P. fluorescens* ATCC 17400 has a higher percentage of proteins involved in bacterial secretion, iron acquisition, and heavy metal detoxification, which may be associated with its significant siderophore production. Additionally, *A. brasilense* Sp245 has the highest number of proteins involved in nitrogen acquisition compared to the other two PGPR, which is consistent with its ability to fix nitrogen due to its nitrogenase-encoding genes *nifD*HK.

3.2. Transcriptomic analysis of PGPR strains in response to root exudates

The gene expression of the three PGPR strains (*Bacillus subtilis* ATCC 6633, *Pseudomonas fluorescens* ATCC 17400, and *Azospirillum brasilense* Sp245) was studied in response to root exudates from rapeseed, pea, and ryegrass. The objective was to identify the specific regulations underlying the distinct behaviors that had previously been reported for these plant-PGPR pairs (Fourneau et al., 2024).

3.2.1. Characterization of bacterial gene expression in response to root exudates

Each PGPR strain was cultivated on two reference media (M9 and LB), as well as on root exudates from rapeseed, pea, and ryegrass – used as the sole nutrient source – until the late exponential growth phase. Bacterial RNA was then extracted, sequenced, and analyzed (Supplementary Table S1). The gene expression profiles for each condition are shown in a heatmap (Fig. 3). For *B. subtilis* ATCC 6633, distinct gene expression profiles are observed for each medium and root exudates, with low variability between replicates except for ryegrass exudates. For *P. fluorescens* ATCC 17400, gene expression in response to the rich LB medium differs from the other conditions, while no clear difference is visible between root exudates and the minimal M9 medium,

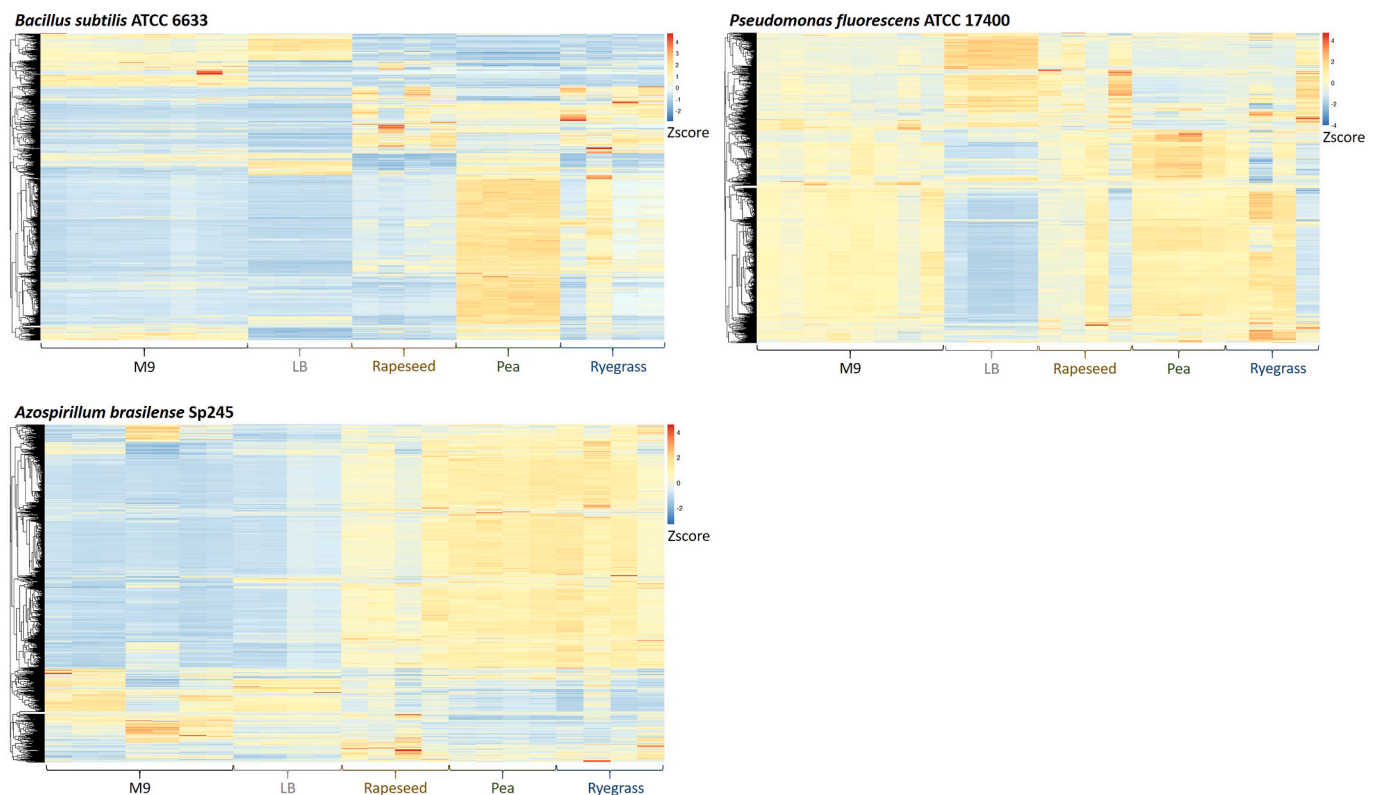


Fig. 3. Heatmaps representing gene expression in the three bacteria when grown on reference media (M9 or LB) or on root exudates from rapeseed, pea, or ryegrass. The colored horizontal lines represent the gene expression levels through Z-score (number of standard deviations from the mean) for each replicate ($n \geq 4$, vertical bands) in each condition.

despite some variability between rapeseed and ryegrass replicates. For *A. brasilense* Sp245, gene expression profiles mainly differ between reference media and root exudates. Gene expression variability was also visualized using principal component analysis, which led to the same conclusions as the heatmap (Supplementary Figure S1). The variability observed between replicates likely reflects that root exudate samples were collected from different campaigns, thus providing insight into biological variability.

Differential gene expression (DGE) analysis was conducted to identify significantly upregulated and downregulated genes in response to root exudates compared to the minimal M9 medium. The rich LB medium was used as an additional control. DGE results are represented by volcano plots, which illustrate the number of differentially expressed genes. Compared to M9, LB modified the expression of 312 genes in *B. subtilis* ATCC 6633, 600 genes in *P. fluorescens* ATCC 17400, and 193 genes in *A. brasilense* Sp245, which is consistent with their distinct composition (Supplementary Figure S2). For *B. subtilis* ATCC 6633, root exudates from rapeseed, pea, and ryegrass downregulate the expression of 240, 732, and 384 genes, respectively, while upregulating 367, 232, and 394 genes (Fig. 4). This suggests that exudates from these three plants induce strong transcriptomic regulation. In contrast, *P. fluorescens* ATCC 17400 regulates far fewer genes, as only 15, 140, and 19 are downregulated, while 54, 14, and 37 genes are upregulated in response to rapeseed, pea, and ryegrass exudates, respectively. Finally, *A. brasilense* Sp245 displays the most important response, as exudates from rapeseed, pea and ryegrass downregulate 467, 995, and 878 genes while upregulating 422, 930, and 807 genes, respectively.

Taken together, these results show that the three PGPR regulate their gene expression differently in response to root exudates from the three plants. Specifically, *B. subtilis* ATCC 6633 and *A. brasilense* Sp245 greatly

modulate their transcriptomes, while *P. fluorescens* ATCC 17400 appears to already express most of the genes required for growth on root exudates when cultivated in M9 with glucose as carbon source.

3.2.2. Comparison of regulated genes in response to root exudates

The differentially expressed genes of each bacterium were compared between conditions to determine the number of regulated genes either common or specific to root exudates from the three plant species (Fig. 5). For *B. subtilis* ATCC 6633, many genes are regulated by all three root exudates. Specifically, the expression of 101 upregulated and 185 downregulated genes constitute a core transcriptome induced by all three plant exudates. Each plant also regulates the expression of specific genes, while some are modulated by only two plant species. In contrast, the core transcriptome of *P. fluorescens* ATCC 17400 results from only 4 upregulated and 9 downregulated genes. Although the number of differentially expressed genes is lower for this PGPR than for the other two, the regulation is largely plant-specific. Finally, an important part of *A. brasilense* Sp245 response is shaped by a core transcriptome resulting from 358 upregulated genes and 411 downregulated genes. Dozens of genes are also regulated by each plant species. Interestingly, 395 and 387 genes are co-regulated by both ryegrass and pea exudates.

The number of differentially expressed genes in response to root exudates and LB medium was also compared. In most cases, the majority of the genes regulated in response to LB are specific to that medium and not shared with exudate response (Supplementary Figure S3). This shows that PGPR responses to root exudates differ substantially from their response to a rich laboratory medium, and involve distinct sets of genes. However, the three PGPR strains use contrasting strategies on exudates: *B. subtilis* ATCC 6633 and *A. brasilense* Sp245 regulate numerous genes, including, for each, an important subset which is

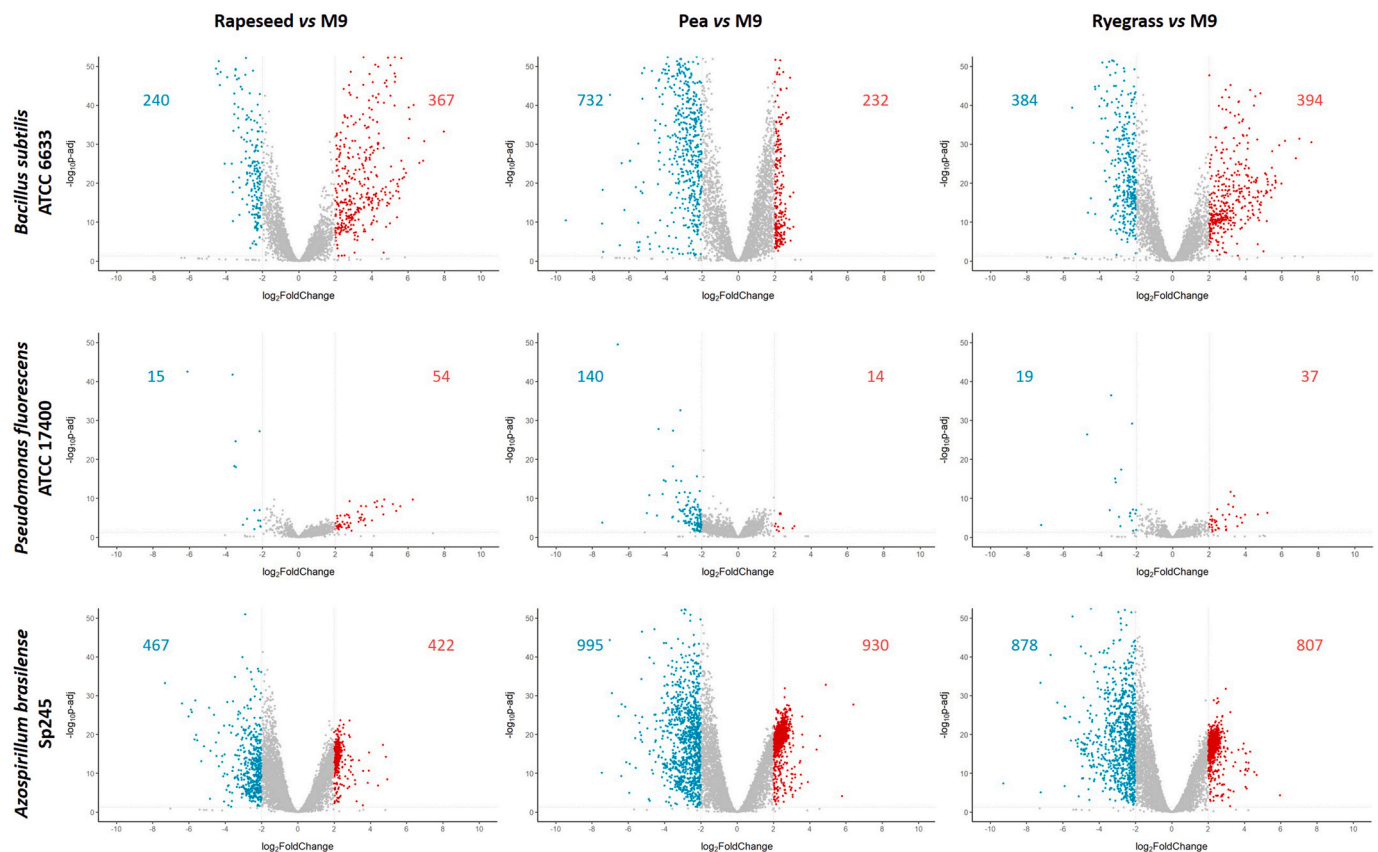


Fig. 4. Volcano plots representing differentially expressed genes in the three bacteria when grown on root exudates from rapeseed, pea, or ryegrass, compared to M9 medium. Gene expression levels ($\log_2$ FoldChange) are plotted against their statistical significance ($-\log_{10}$ adjusted p-value) and reveal the number of significantly downregulated and upregulated genes (blue and red dots, respectively).

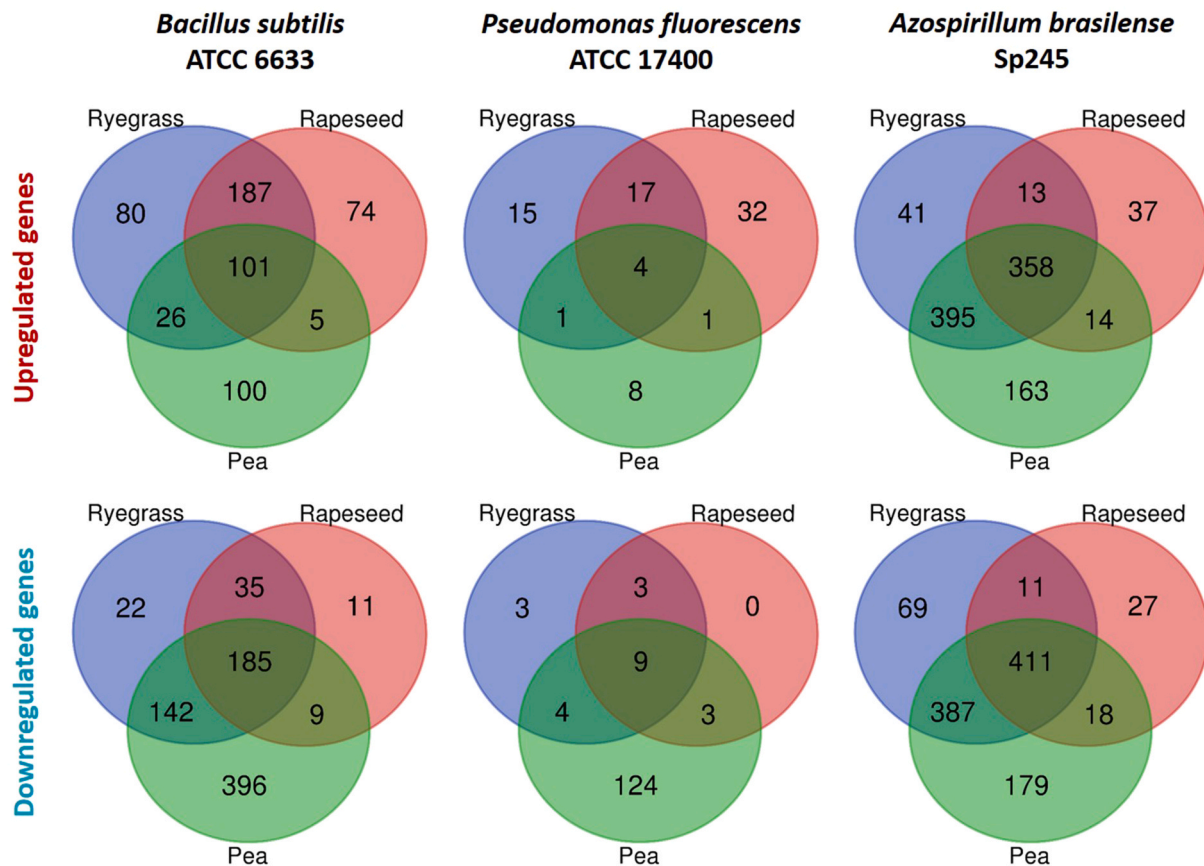


Fig. 5. Venn diagrams representing the number of regulated genes that are common or specific to the bacterial response to root exudates from rapeseed, pea, and ryegrass, compared to M9 medium.

common to all three exudates (i.e., the core transcriptome), while *P. fluorescens* ATCC 17400 regulates fewer genes with a more plant-specific response.

3.2.3. Functions of regulated genes in response to root exudates

The functions of the differentially expressed genes of all three bacteria were identified using the COG database. The functions of upregulated and downregulated genes in the three PGPR strains in response to LB medium and to root exudates from rapeseed, pea, and ryegrass are shown in [Supplementary Figure S4](#). Thereafter, we describe the functions of bacterial genes regulated via the core transcriptome induced by all three plants, as well as the genes specifically regulated by exudates from each plant species ([Fig. 6](#), [Supplementary Table S2](#)).

For *B. subtilis* ATCC 6633, the core transcriptome results from the upregulation of 101 genes, primarily involved in carbohydrate, lipid and amino acid transport and metabolism, as well as energy production and conversion. These comprise genes involved in the transport of sugars (*yesP*), arabinooligosaccharides (*araN*, *araQ*), glucarate (*exuT*) and inositol (*iolF*), as well as in the catabolism of maltose (*malH*), arabinose (*araA*, *araD*), lysine (*kamA*) and rhamnogalacturonan (EO946_RS03870). This suggests that such molecules are present in all three plant root exudates. Additionally, 15 genes involved in sporulation are upregulated. The 185 genes that are downregulated through the core transcriptome are mostly involved in inorganic ion, nucleotide and amino acid transport and metabolism, as well as translation. These include genes involved in the biosynthesis of serine (*serA*), glutamine (*glnA*), methionine (*metE*, *yxjG*) and lysine (*dapH*), which may be less necessary if these amino acids are already supplied by exudates, compared to M9 medium.

B. subtilis ATCC 6633 specifically regulates its gene expression in response to root exudates from each plant species. Indeed, rapeseed

exudates specifically stimulate the expression of 74 genes mainly involved in amino acid and carbohydrate transport and metabolism. These include genes involved in the catabolism of alanine (*ald*), glycine (*gcvPA*, *gcvPB*, *gcvT*) and lysine (*gabT*), as well as in the transport of oligopeptides (*appF*, *dtpT*), arginine/ornithine (*rocC*), aldouronate (*lplB*) and even degraded pectin products imported through a permease (*kdgT*). Interestingly, three genes upregulated by rapeseed exudates are involved in defense mechanisms. These include a lanthionine synthetase involved in lantibiotic synthesis (EO946_RS16955), and two lantibiotic transporters (EO946_RS16940, EO946_RS16960), which ensure resistance for the producing strain.

Pea exudates specifically stimulate the expression of 100 genes in *B. subtilis* ATCC 6633 mainly involved in carbohydrate and amino acid transport and metabolism, such as tryptophan biosynthesis (EO946_RS21090, EO946_RS21095) and ribose transport (*rbsA*). Genes encoding stress proteins (*gsiB*, *nhaX*) were also found upregulated. Pea exudates downregulate the expression of 396 genes, most of which are involved in translation, transcription, and amino acid transport and metabolism, such as the biosynthesis of cysteine (*cysK*), histidine (*hisC*), glutamate (*gltA*), leucine/isoleucine (*leuA*, *leuB*, *leuC*, *leuD*), valine/isoleucine (*ilvA*, *ilvC*, *ilvN*), lysine (*dapA*), arginine (*argF*, *argG*, *argH*), methionine (*yxjG*), tryptophan (*trpA*) and serine (*serC*). The expression of six flagellar or motility genes (*yjfb*, *flhA*, *flhF*, *flhW*, *swrA*, *flgC*) and two bacteriocin genes (EO946_RS16950, EO946_RS18850) was also down-regulated in response to pea exudates.

Finally, ryegrass exudates specifically stimulate the expression of 80 genes in *B. subtilis* ATCC 6633, many of which are involved in cell envelope biogenesis. More precisely, ten sporulation genes are upregulated. Ryegrass exudates also repress the expression of 22 genes, including three genes involved in motility (*flhE*, *flhQ*, *flhB*).

The core transcriptome of *P. fluorescens* ATCC 17400 results from the

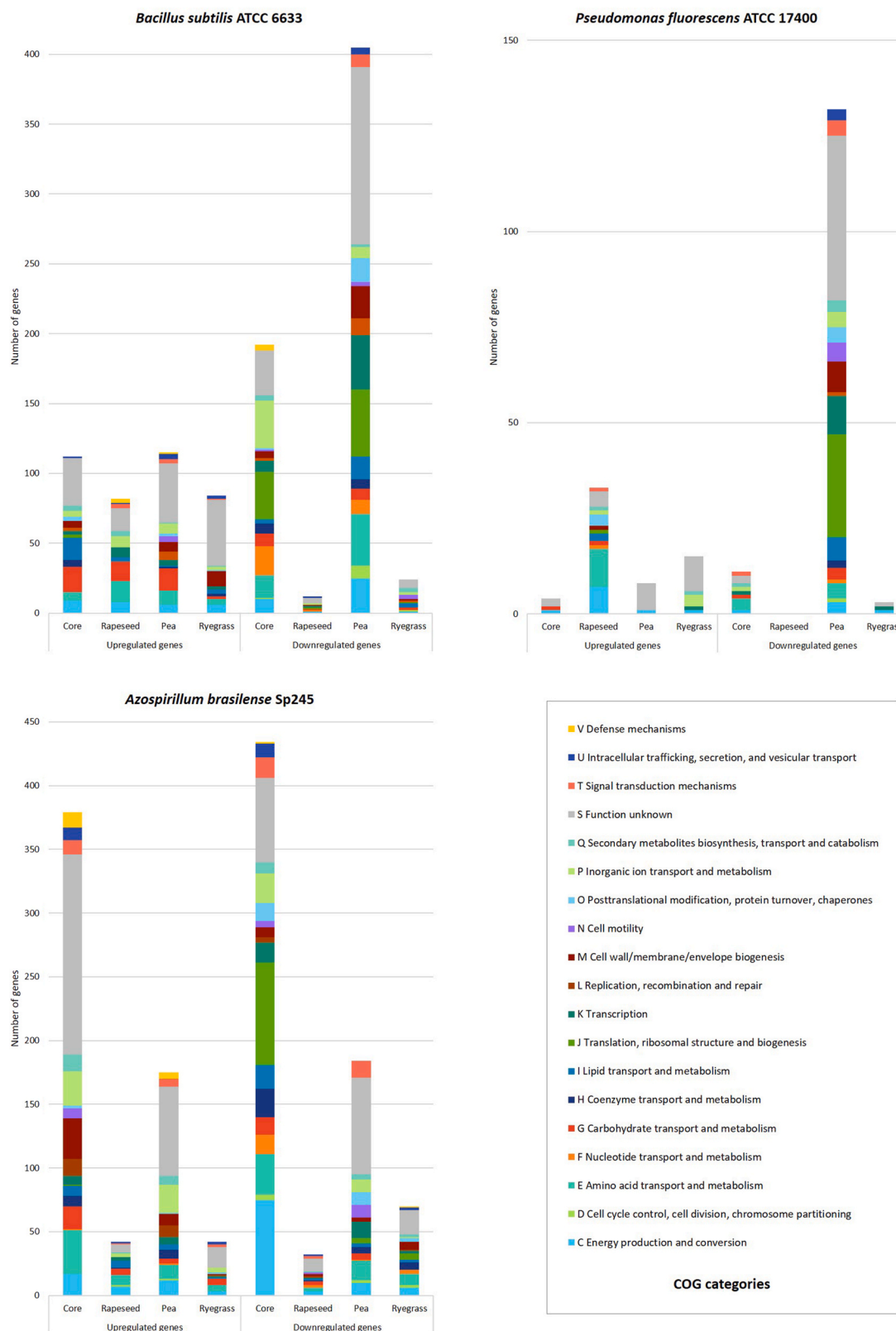


Fig. 6. COG functional categories of bacterial genes regulated in response to root exudates compared to M9 medium. One gene may be associated with multiple categories. Core: genes regulated by all three plant exudates and expressed through the core transcriptome. Rapeseed, Pea, Ryegrass: genes specifically regulated by each plant's exudates.

Table 2

Overview of the main functions of interest regulated by root exudates in the three bacteria. Up arrows: upregulated functions, down arrows: downregulated functions.

PGPR strain	Core transcriptome	Plant-specific genes		
		Rapeseed	Pea	Ryegrass
<i>Bacillus subtilis</i> ATCC 6633	↑ Amino acid, sugar, and plant polysaccharide catabolism and transport ↑ Sporulation ↓ Amino acid biosynthesis	↑ Amino acid catabolism and transport ↑ Lantibiotic production	↑ Amino acid and carbohydrate metabolism and transport ↑ Stress response ↓ Amino acid biosynthesis ↓ Motility	↑ Sporulation ↓ Motility
<i>Pseudomonas fluorescens</i> ATCC 17400	↓ Amino acid biosynthesis	↑ Amino acid metabolism and transport	↓ Nucleotide sugar biosynthesis ↓ Motility	↑ Inorganic ion metabolism
<i>Azospirillum brasilense</i> Sp245	↑ Amino acid, sugar, and plant polysaccharide catabolism and transport ↑ Efflux ↑ Nitrogen fixation ↓ Amino acid biosynthesis	↑ Amino acid metabolism and transport ↓ Type VI secretion system	↑ Amino acid metabolism and transport ↑ Resistance proteins ↑ Nitrogen fixation ↑ Phosphate solubilization ↓ Amino acid biosynthesis ↓ Motility	↑ Amino acid and carbohydrate metabolism and transport ↓ Amino acid metabolism

regulation of a very small number of genes, including three that are downregulated and involved in amino acid transport and metabolism, such as a methionine biosynthesis gene (*metE*). However, several genes are specifically regulated in response to each plant species. Indeed, rapeseed exudates stimulate the expression of 32 genes, ten of which are involved in amino acid transport and metabolism, such as glycine betaine and proline transport (*opuAC*, *choX*), as well as leucine biosynthesis (*leuA*). Ryegrass exudates specifically stimulate the expression of 15 genes, three of which are involved in inorganic ion transport and metabolism, such as bacterioferritin (BG51_08760). In contrast, pea exudates specifically downregulate the expression of 124 genes mainly involved in translation, transcription, and cell envelope biogenesis. Two genes involved in nucleotide sugar biosynthesis (*fcl*, *ddhC*) are also downregulated. Interestingly, the expression of five genes involved in motility and chemotaxis (*cheV*, *cheY*, *flgM*, *fliE*, *fliS*) is repressed by pea exudates.

For *A. brasilense* Sp245, the core transcriptome results from the upregulation of 358 genes, primarily involved in amino acid, inorganic ion and carbohydrate transport and metabolism, as well as cell envelope biogenesis. These include genes involved in plant polysaccharide degradation such as starch (Sp245p_RS12700, Sp245p_RS29245), xylan (Sp245p_RS24940) and pectic acid (Sp245p_RS16350). Other genes are involved in peptide transport (Sp245p_RS24360, Sp245p_RS27445), efflux proteins (*oprM*, *emrA*, Sp245p_RS24100) as well as nitrogen fixation (*nif* genes). Moreover, the 411 genes downregulated through the core transcriptome are mainly involved in translation, energy production and conversion, as well as amino acid, inorganic ion and coenzyme transport and metabolism. In particular, these include genes involved in the biosynthesis of valine/isoleucine (*ilvC*, *ilvD*), serine (*serA*), cysteine (*cysB*, *cysK*), arginine (*argC*, *argG*), leucine/isoleucine (*leuB*, *leuD*), lysine (*dapD*) and glutamine (*glnA*), as well as glycolysis and gluconeogenesis genes (*eno*, *glpX*).

A. brasilense Sp245 also specifically regulates gene expression in response to each plant root exudates. In particular, rapeseed exudates specifically stimulate the expression of 37 genes primarily involved in amino acid transport and metabolism as well as energy production and conversion, such as oxalate catabolism (*frc*, Sp245p_RS31845). Moreover, three of the 27 downregulated genes are involved in type VI secretion system (Sp245p_RS14660, Sp245p_RS14665, Sp245p_RS14670).

Pea root exudates specifically upregulate the expression of 163 genes in *A. brasilense* Sp245 mainly involved in inorganic ion and amino acid transport and metabolism, as well as energy production and conversion. These include genes involved in arginine transport (Sp245p_RS2999), glycolysis (*ttuE*), and antibiotic and multidrug resistance proteins (*fss*, Sp245p_RS31570). Some of the upregulated genes are involved in plant growth-promoting traits, such as nitrogen fixation (*nifB*, *nifE*, *nifQ*, *nifW*)

and pyrroloquinoline quinone biosynthesis (*pqqB*), which is responsible for phosphate solubilization. The 179 genes specifically downregulated in response to pea exudates are primarily involved in amino acid transport and metabolism, transcription, and signal transduction mechanisms. These include genes involved in the biosynthesis of leucine (*leuA*), glutamine (Sp245p_RS08330), tryptophan (*trpG*), lysine (Sp245p_RS00590), arginine (*argB*) and unsaturated fatty acids (*tesA*), as well as ribose transport (Sp245p_RS23160). Pea exudates also specifically repress the expression of ten motility genes (e.g., *flgB*, *flgC*, *flgF*, *fliL*, *fliX*).

Finally, ryegrass exudates specifically stimulate the expression of 41 genes in *A. brasilense* Sp245, of which ten are involved in amino acid and carbohydrate transport and metabolism, including galactofuranose transport (*ytfQ*, *yjfF*). Ryegrass exudates also repress the expression of 69 genes, including nine involved in amino acid transport and metabolism, such as the biosynthesis of glutamate (*gltB*) and arginine (*argH*), as well as glycine degradation (*gcvH*, *gcvP*, *gcvPA*).

Overall, the functional annotation reveals that root exudates from all three plants stimulate the expression of bacterial genes involved in the catabolism and transport of amino acids and carbohydrates, including plant polysaccharides (Table 2). Root exudates also repress the expression of genes involved in amino acid biosynthesis. In addition to this core transcriptome, each plant species modifies the expression of specific PGPR genes, while PGPR strains exhibit unique responses, such as sporulation in *B. subtilis* ATCC 6633, or plant-growth promoting traits in *A. brasilense* Sp245.

4. Discussion

4.1. PGPR display two distinct adaptation strategies in response to root exudates

The objective of this study was to investigate the genetic responses of three PGPR strains to root exudates from three different plants. First, a comparative genomic analysis was conducted and highlighted the specific characteristics of the genomes of *Bacillus subtilis* ATCC 6633, *Pseudomonas fluorescens* ATCC 17400, and *Azospirillum brasilense* Sp245, in relation to their potential as PGPR. In particular, more than half of the proteome of these three strains encodes plant growth-promoting traits (PGPTs), such as tolerance to abiotic stress or plant-derived substrate usage. To be used as efficient bioinoculants, these PGPR must possess rhizocompetence traits, i.e. characteristics conferring an advantage in rhizosphere colonization. These include motility and chemotaxis, utilization of root exudates as nutrients, biofilm formation, and competition with other microorganisms (Kaur et al., 2017; Patten, 2022; Santoyo et al., 2021). In this context, our transcriptomic analysis aimed to identify biological functions that are genetically regulated in *B. subtilis*

ATCC 6633, *P. fluorescens* ATCC 17400, and *A. brasilense* Sp245 in response to root exudates from rapeseed, pea, and ryegrass. We specifically focused on rhizocompetence functions such as amino acid and carbohydrate transport and metabolism, cell motility, and defense mechanisms, to determine whether they were differentially triggered by exudates from different plant species. It is important to note that the functions of several genes regulated by root exudates remain unknown due to the unannotated portion of bacterial genomes.

The PGPR strains regulated different numbers of genes during their growth on rapeseed, pea, and ryegrass exudates. In response to each plant's exudates, *B. subtilis* ATCC 6633 and *A. brasilense* Sp245 modified the expression of at least 600 and 800 genes, respectively, whereas *P. fluorescens* ATCC 17400 only regulated between 50 and 150 genes, depending on the plant. Although the higher number of genes regulated by *A. brasilense* Sp245 may be attributed to its larger genome (7.7 Mb) compared to *B. subtilis* ATCC 6633 (4 Mb), the number of genes regulated by *P. fluorescens* ATCC 17400 in response to root exudates was remarkably lower, despite its 6.3 Mb genome. The three PGPR strains studied therefore display two distinct adaptation strategies: *B. subtilis* ATCC 6633 and *A. brasilense* Sp245 greatly adjust their gene expression in response to root exudates, whereas *P. fluorescens* ATCC 17400 appears to be already adapted to them due to its constitutive functions. This could be explained by the differential gene expression being compared to the M9 medium, in which *P. fluorescens* ATCC 17400 could already express these genes. Nevertheless, this strain seems preadapted to efficiently utilize a wide range of compounds present in root exudates, thereby requiring minimal transcriptional reprogramming. Indeed, *Pseudomonas* species are well-known for their ability to thrive in diverse environments thanks to their broad metabolisms that fit to various ecological conditions (Nikolaidis et al., 2020; Silby et al., 2011). In contrast, the high number of genes regulated by *A. brasilense* Sp245 may be linked to its six chromids and plasmids, which provide additional sets of functions for responding to exudates (e.g. amino acid transport) and thus enhance its adaptability (Orlandini et al., 2014).

4.2. PGPR core transcriptomes reveal a root exudate core metabolome

The functions of differentially expressed PGPR genes were then studied, with an initial focus on those that were regulated by all three plants' root exudates. The core transcriptomes of *B. subtilis* ATCC 6633 and *A. brasilense* Sp245 resulted from the regulation of hundreds of genes. These include upregulated genes involved in the catabolism and transport of amino acids (e.g. lysine), sugars (e.g. maltose, arabinose), and plant polysaccharides (e.g. rhamnogalacturonan, starch, xylan). This reflects the uptake and utilization of these molecules from root

exudates for bacterial growth. In fact, amino acids and sugars are primary metabolites that are very often studied and described in root exudates, while root mucilage polysaccharides can also be present (Badri and Vivanco, 2009; Vives-Peris et al., 2020). In addition, the core transcriptomes of all three PGPR strains resulted from the down-regulation of amino acid biosynthesis genes (e.g. serine, glutamine, methionine), which may no longer be needed if these amino acids are directly taken up by bacteria from root exudates.

Therefore, these results enabled us to deduce the compounds in rapeseed, pea, and ryegrass exudates detected by PGPR strains as they induced a transcriptional response. Table 3 thus presents the amino acids and sugars whose: i) catabolism was stimulated, ii) transport was stimulated, and/or iii) biosynthesis was repressed. The resulting list highlights potential plant-specific compounds of root exudates, although their identification may depend on the response of each strain (e.g. glutamate). Table 3 also includes a core metabolome shared by root exudates of all three plant species, identified from the core transcriptomes. Indeed, McLaughlin et al. (2023) have identified core metabolites in root exudates from three phylogenetically distinct plant species (*Arabidopsis thaliana*, *Brachypodium distachyon*, and *Medicago truncatula*), including amino acids such as glutamine, leucine, serine and valine, which may also be present in rapeseed, pea, and ryegrass exudates, according to our results (Table 3). Thus, the root exudate core metabolome may be responsible for the recruitment of a core microbiome by all plant species, mainly by providing a nutrient source for microbial growth (McLaughlin et al., 2023; Tsai et al., 2025). Metabolomic analyses will aim to confirm the inferred composition of exudates.

4.3. Different bacterial functions are regulated by plant-specific root exudates

In addition, numerous PGPR genes were regulated by only one plant, highlighting the specific effect of each plant species' exudates. Previously, we observed distinct physiological behaviors of *B. subtilis* ATCC 6633, *P. fluorescens* ATCC 17400, and *A. brasilense* Sp245 in response to root exudates from rapeseed, pea, and ryegrass. More precisely, rapeseed exudates were the most attractive and induced the fastest growth, pea exudates allowed the highest biomass production, while ryegrass exudates were the least efficient (Fourné et al., 2024). Taken together, these genetic and physiological responses support the hypothesis that, in addition to the core metabolome, species-specific exudates enable the plant to select specific microorganisms in the rhizosphere. In particular, rapeseed exudates stimulated the expression of amino acid metabolism and transport genes, while they also induced a strong chemotaxis

Table 3
Amino acids and sugars detected by *Bacillus subtilis* ATCC 6633 (Bs), *Pseudomonas fluorescens* ATCC 17400 (Pf), and *Azospirillum brasilense* Sp245 (Ab) in rapeseed, pea, and ryegrass root exudates.

Root exudates of all three plants (core metabolome)	Plant-specific root exudates		
	Rapeseed	Pea	Ryegrass
Lysine (Bs, Ab)	Alanine (Bs)	Histidine (Bs)	Glutamate (Ab)
Serine (Bs, Ab)	Glycine (Bs, Pf)	Glutamate (Bs)	
Glutamine (Bs, Ab)	Proline (Pf)	Tryptophan (Bs, Ab)	
Methionine (Bs, Pf)			
Cysteine (Ab)			
Arginine (Ab)			
Leucine (Ab)			
Isoleucine (Ab)			
Valine (Ab)			
Inositol (Bs)		Ribose (Bs)	Galactofuranose (Ab)
Maltose (Bs)			
Arabinose (Bs)			
Rhamnogalacturonan (Bs)			
Starch (Ab)			
Xylan (Ab)			

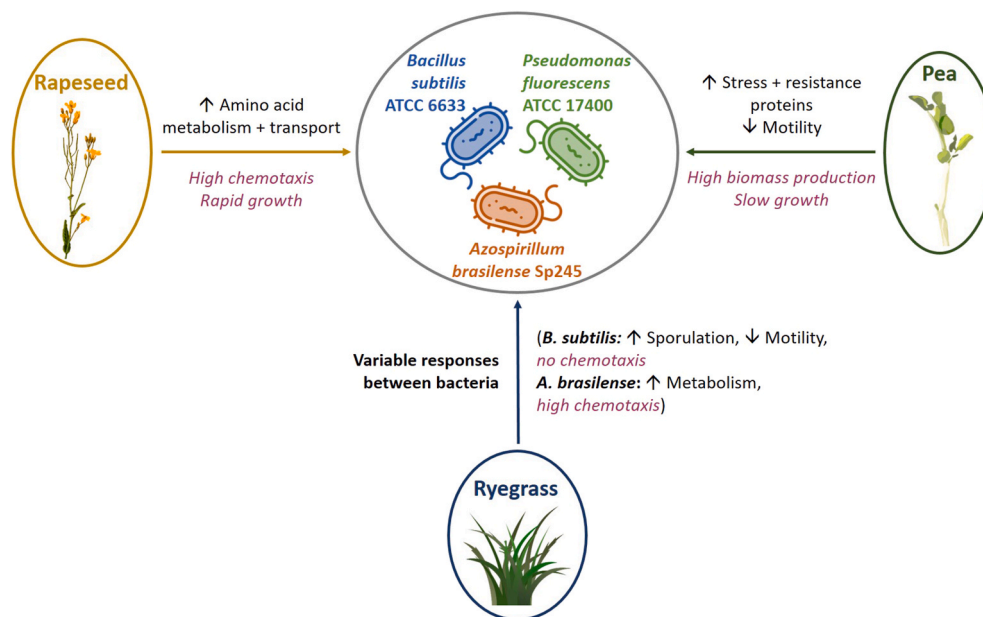


Fig. 7. Overview of the bacterial responses specifically induced by root exudates from rapeseed, pea, and ryegrass. Up and down arrows indicate functions of upregulated and downregulated genes determined in this work; purple italics indicate physiological responses determined in previous work (Fourneau et al., 2024).

response and a rapid growth for the three PGPR strains (Fig. 7). Rapeseed exudates thus contain chemoattractants and nutrients that are rapidly catabolized, including amino acids, which serve as a source of both carbon and nitrogen, and have an estimated half-life of a few hours in soil due to microbial utilization (Moe, 2013). Pea exudates specifically stimulated the expression of stress and resistance proteins in *B. subtilis* ATCC 6633 and *A. brasilense* Sp245, while simultaneously repressing the expression of motility genes in the three strains studied. Additionally, despite a high biomass production, bacterial growth on pea exudates was slow (Fourneau et al., 2024). The repression of motility genes is surprising, since a positive chemotaxis response was previously observed, but this could be explained by the different growth times (i.e. late exponential phase for transcriptomic analyses vs early bacterial response for chemotaxis assays). Moreover, the expression of stress proteins and slow bacterial growth could be explained by the presence of antimicrobial molecules such as soyasaponins, which have been specifically identified in root exudates of legumes, including pea (Calabrese et al., 2023; Seitz et al., 2023). Finally, ryegrass exudates induced both genetic and physiological variable responses among PGPR strains, with differences in the regulation of gene functions, as well as particularly variable chemotaxis responses. In particular, *A. brasilense* Sp245 appears to benefit from ryegrass exudates, as they stimulate its chemotaxis and metabolism, while *B. subtilis* ATCC 6633 seems to suffer from them as they repress its chemotaxis and motility, and trigger its sporulation (Fig. 7). Ryegrass may thus be more selective in recruiting its rhizosphere microbiota by favoring certain bacteria over others, such as Pseudomonadota (Cao et al., 2024; Chen et al., 2016).

Our observations show that distinct bacterial species can respond differently to specific root exudates. Indeed, *B. subtilis* ATCC 6633 upregulated the expression of many sporulation genes in response to root exudates from rapeseed, pea, and ryegrass (Table 2). Although this process could be caused by nutritional stress, sporulation in *B. subtilis* has been observed in response to *A. thaliana* roots, suggesting that root exudates promote bacterial sporulation (Charron-Lamoureux and Beauregard, 2019). This may explain why *Bacillus* cells struggle to multiply when applied to plants in agricultural conditions (Charron-Lamoureux and Beauregard, 2019). Furthermore, *B. subtilis* ATCC 6633 specifically upregulated genes involved in lantibiotic production in response to rapeseed exudates. Indeed, *B. subtilis* ATCC 6633 is able to synthesize lantibiotic antibiotics such as subtilin and nisin,

which permeabilize bacterial membranes of sensitive bacteria, resulting in pore formation and bacterial death (Parisot et al., 2008). Thus, *B. subtilis* ATCC 6633 may specifically produce antimicrobial peptides in response to rapeseed exudates, which appear to be particularly rich in nutrients, in order to compete with other bacteria for this favorable ecological niche. Concerning *A. brasilense* Sp245, the expression of certain nitrogen fixation genes was upregulated in response to all three root exudates (Table 2), indicating that this PGPR is required by all three plants. However, between the three strains studied, *A. brasilense* Sp245 is the only one capable of synthesizing nitrogenase, responsible for nitrogen fixation (Giri et al., 2025). Interestingly, pea exudates specifically stimulated the expression of additional nitrogen fixation genes, as well as one gene involved in phosphate solubilization, in *A. brasilense* Sp245. Although pea normally obtains bioavailable nitrogen through its symbiosis with rhizobia, our results may be explained by the plant's need to compensate for the absence of this symbiosis under hydroponic conditions (Franché et al., 2009). Besides root exudates, bacteria like *B. subtilis* can also stimulate nitrogen fixation by *A. brasilense* when co-inoculated (Galindo et al., 2025). In contrast, *P. fluorescens* ATCC 17400 exhibited a very different genetic response to root exudates by regulating the expression of fewer genes and therefore, fewer functions. The specific transcriptomic signatures identified in this study should be compared with data from soil- and field-based studies that more accurately reflect rhizosphere conditions. While some responses may be specific to *in vitro* conditions (e.g. stimulation of nitrogen fixation by pea exudates), others are likely to occur *in situ* (e.g. induction of metabolic and transport genes in response to root exudates, sporulation in *B. subtilis*).

5. Conclusion

In conclusion, this study highlighted the different genetic responses of *B. subtilis* ATCC 6633, *P. fluorescens* ATCC 17400, and *A. brasilense* Sp245 to root exudates from rapeseed, pea, and ryegrass. Two distinct bacterial adaptation strategies were identified: the regulation of hundreds of genes in *B. subtilis* ATCC 6633 and *A. brasilense* Sp245 reflects a strong adaptability to root exudates, while the regulation of only dozens of genes by *P. fluorescens* ATCC 17400 shows that most of the necessary functions are constitutively expressed. The genes regulated by all three plants and expressed through the core transcriptome are primarily

involved in metabolism, and their regulation appears to respond to a root exudate core metabolome. However, each plant species also modulated the expression of specific PGPR genes associated with distinct functions, such as amino acid metabolism for rapeseed and defense mechanisms for pea, consistent with previously observed physiological responses (Fourneau et al., 2024). In sustainable agriculture, rhizosphere engineering represents a novel strategy based on the modulation of rhizosphere components, including both plants and microorganisms (Dessaux et al., 2016; Solanki et al., 2024). In particular, rhizosphere microbiota engineering includes the application of specific microorganisms (e.g. bioinoculants, synthetic communities), and the modulation of root exudates to help plants recruit certain microorganisms (Bano et al., 2021; Park et al., 2023). Therefore, using specific root exudates, directly as amendments or indirectly through intercropping, could attract and maintain beneficial microorganisms such as PGPR in the rhizosphere (Barbosa et al., 2020; Li et al., 2023; Yang et al., 2023). Taken together, our results suggest that using certain root exudates, such as those from rapeseed, could enhance bioinoculant establishment, as they stimulate bacterial metabolism, chemotaxis, and growth. The selection of the PGPR bioinoculant strain should also be carefully considered, since, for example, ryegrass exudates appeared to favor *A. brasilense* Sp245 over *B. subtilis* ATCC 6633. Further research will aim to identify which particular compounds or groups of compounds are responsible for the specific bacterial responses using metabolomics, as well as validate these results in field experiments and study other microorganisms (Calabrese et al., 2023; Escolà Casas and Matamoros, 2021; Poppeliers et al., 2023).

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CRedit authorship contribution statement

Eulalie Fourneau: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Baptiste Barbault:** Formal analysis, Investigation, Visualization, Writing – review & editing. **Mélissa Pannier:** Investigation, Methodology, Writing – review & editing. **Barbara Pawlak:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Josselin Bodilis:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.rhisph.2026.101293>.

Data availability

Data will be made available on request.

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