MPMI Vol. 36, No. 9, 2023, pp. 536–548, https://doi.org/10.1094/MPMI-10-22-0223-CR

*CURRENT REVIEW*

# Molecular Mechanisms of *Pseudomonas*-Assisted Plant Nitrogen Uptake: Opportunities for Modern Agriculture

**Stefan Sanow,[1,2] Weiqi Kuang,[3] Gabriel Schaaf,[4] Pitter Huesgen,[5] Ulrich Schurr,[1] Ute Roessner,[6] Michelle Watt,[2] and Borjana Arsova[1,†]**

[1] Institute for Bio- and Geosciences, Plant Sciences (IBG-2), Forschungszentrum Juelich GmbH, Germany
[2] School of BioSciences, Faculty of Science, The University of Melbourne, Parkville, 3010 Victoria, Australia
[3] College of life and Environmental Sciences, Hunan University of Arts and Science, China
[4] Institute of Crop Science and Resource Conservation, University of Bonn, 53115 Bonn, Germany
[5] Central institute for Engineering, Electronics and Analytics (ZEA-3), Forschungszentrum Juelich GmbH, Germany
[6] Research School of Biology, The Australian National University, Acton, 2601 Australian Capital Territory, Australia

Accepted for publication 27 March 2023.

***Pseudomonas* spp. make up 1.6% of the bacteria in the soil and are found throughout the world. More than 140 species of this genus have been identified, some beneficial to the plant. Several species in the family Pseudomonadaceae, including *Azotobacter vinelandii* AvOP, *Pseudomonas stutzeri* A1501, *Pseudomonas stutzeri* DSM4166, *Pseudomonas szotifigens* 6HT33bT, and *Pseudomonas* sp. strain K1 can fix nitrogen from the air. The genes required for these reactions are organized in a nitrogen fixation island, obtained via horizontal gene transfer from *Klebsiella pneumoniae*, *Pseudomonas stutzeri*, and *Azotobacter vinelandii*. Today, this island is conserved in *Pseudomonas* spp. from different geographical locations, which, in turn, have evolved to deal with different geo-climatic conditions. Here, we summarize the molecular mechanisms behind *Pseudomonas*-driven plant growth promotion, with particular focus on improving plant performance at limiting nitrogen (N) and improving plant N content. We describe *Pseudomonas*-plant interaction strategies in the soil, noting that the mechanisms of denitrification, ammonification, and secondary metabolite signaling are only marginally explored. Plant growth promotion is dependent on the abiotic conditions and differs at sufficient and deficient N. The molecular controls behind different plant responses are not fully elucidated. We suggest that superposition of transcriptome, proteome, and metabolome data and their integration with plant phenotype development through time will help fill these gaps. The aim of this review is to summarize the knowledge behind *Pseudomonas*-driven nitrogen fixation and to point to possible agricultural solutions.**

[†]Corresponding author: B. Arsova; b.arsova@fz-juelich.de

**Funding:** This work was funded by the Deutsche Forschungsgemeinschaft grants EXC 2048 (CEPLAS), EXC 2070–390732324 (PhenoRob), and grant 491111487 to fund open access publication. B. Arsova and U. Schurr were supported by the Helmholtz-Gemeinschaft "Changing Earth" Topic program. M. Watt was supported through University of Melbourne Botany Foundation by holding the Adrienne Clarke Chair of Botany. S. Sanow received support from the Forschungszentrum Jülich through the Jülich-University of Melbourne Post-Graduate Academy (JUMPA) program.

The author(s) declare no conflict of interest.



*Keywords*: biological nitrogen fixation, molecular mechanism, N-fixation, plant growth–promoting bacteria, *Pseudomonas*

Synthesized fertilizers containing nitrogen (N) have become obligatory in today's agriculture to meet global food demand (Erisman et al. 2008). N fertilizer production is one of the most important innovations for humanity, allowing us to feed an increasing world population. A conservative study estimates that a majority of today's population (7.75 billion) relies on mineral fertilizer (Fig. 1) (Erisman et al. 2008).

However, mineral fertilizer is a double-edged sword creating a number of problems inherent to its production and use. First, N fertilizer production by the Haber-Bosch process is energy intensive and currently still driven by fossil fuels, resulting in greenhouse gas emissions (Smith et al. 2020). Second, in addition to the growing price of fertilizer, a looming problem is excessive nitrogen use that can lead to nitrate leaching into ground water or, depending on soil pH, redox potential, and microbial activity, to $NH_3$ or $N_2O$ emissions causing N losses and environmental pollution (Hirel et al. 2011; Klimczyk et al. 2021; Padilla et al. 2018; Ravishankara et al. 2009). Thus, governments have started to restrict application of N fertilizers (https://www.bmel.de/DE/themen/landwirtschaft/pflanzenbau/ackerbau/ernte2020.html, accessed March 28, 2023). Recent global events like the pandemic, the Russian invasion of Ukraine, and the consequent rise of energy prices by 80% during 2021 not only increased fertilizer prices (Ben Hassen and El Bilali 2022), but, more importantly, affected the fertilizer export chains. This, in turn, threatens the productivity of agriculture in other parts of the world (Jagtap et al. 2022; Mustafa 2022). In the future, the invasion of Ukraine is expected to put further pressure on fertilizer availability (Jagtap et al. 2022).

Restricted N fertilizer use may eventually result in lower yields in existing farming systems with today's crop cultivars and soil management practices. An estimate from the European Union is that the caps led to a 10 to 15.9% yield reduction in wheat over the past six years (BMEL). This will cause less food production and increased prices unless new reliable solutions are found through more nutrient-efficient plant varieties, new farming practices, or new biological N-fixation associations that are reliable across a variety of field conditions.

N is a crucial nutrient for plants, incorporated as the main building block of amino acids, proteins, and many secondary

metabolites. In most agricultural systems, N deficiency plays a major role in plant growth limitation (Bakkou 2011). It is mainly available to the plant in two distinct forms, ammonium ($NH_4^+$) or nitrate ($NO_3^-$). Ammonium is be taken up by plants via the AMT/MEP/Rh-type (ammonium transport system, methylammonium permeases, and Rhesus protein) ammonium transport system. Studies in *Arabidopsis* place most AMTs in the root, contributing to ammonium uptake. One has been found in pollen and one, AMT2.1, contributes to xylem loading and root to shoot ammonium transport (Bindel and Neuhäuser 2021; Giehl et al. 2017). In rice, AMT1.1 and AMT1.3 contribute to ammonium uptake under limited nitrogen conditions and, in the case of AMT1.3, also under sufficient nitrogen conditions (Ranathunge et al. 2014). While ammonium can be directly assimilated to glutamine in the root, nitrate is first transported to the shoot and reduced to ammonium by nitrate- and nitrite- reductase. Nitrate can be taken up by plants via the nitrate transport system (Bock and Wagner 2001; Daims et al. 2015). Among the multiple nitrate transporters, we mention NRT2.1 as an important component of the inducible high-affinity transport system for $NO_3^-$ in the root (Cerezo et al. 2001; Filleur et al. 2001; Li et al. 2007; Trinh et al. 2018). NRT2.1 is induced by $NO_3^-$, but strongly re-

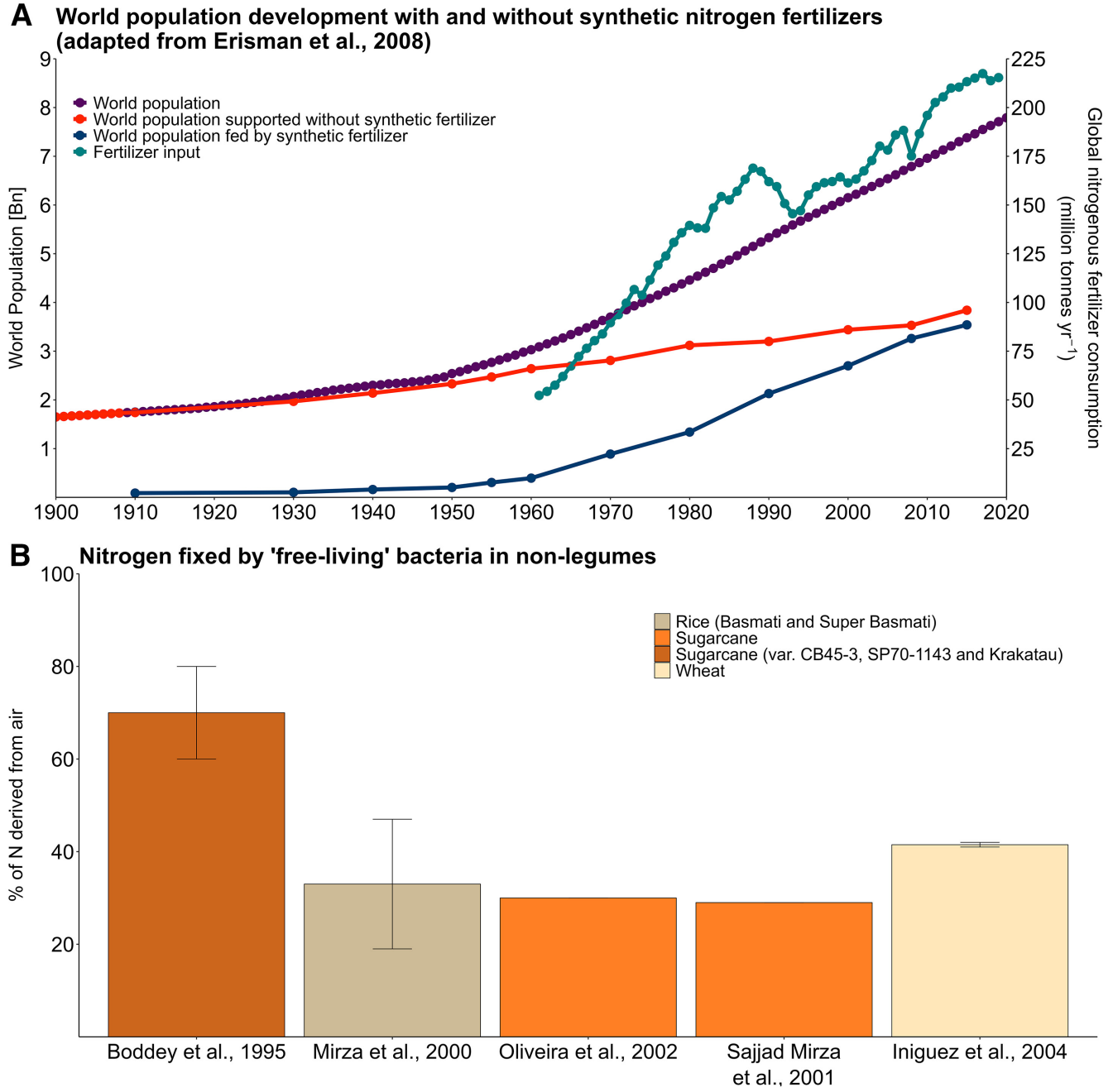


**Fig. 1. A,** World population development with and without the support of synthetic fertilizer during the 20th century, as calculated, simulated, and estimated by Erisman et al. 2008 and the United States Department of Agriculture. The purple line represents world population development, while the red line represents the estimated global population that could be fed without the invention of reactive nitrogen from the Haber-Bosch process. The blue line provides the estimated global population that is fed by synthesized nitrogen. The global consumption of nitrogenous fertilizer in million ton per year is represented in teal. **B,** Nitrogen fixed by 'free-living' bacteria in non-legumes, especially in rice, wheat, and sugarcane variants. Free-living bacteria used in the studies involve *Acetobacter* spp., *Azospirillum* spp., *Burkholderia* spp., *Enterobacter* sp., *Herbaspirillum* spp., and *Klebsiella* sp. Data shown are the average percentages of N content in plants derived from the air via biological nitrogen fixation (BNF) of the bacteria. Error bars are the standard error, representing the range of contribution to total N content via BNF data from Boddey et al. 1995, Mirza et al. 2000, Sajjad Mirza et al. 2001, Oliveira et al. 2002, and Iniguez et al. 2004.

pressed by high N supply to the plant (Filleur et al. 2001; Girin et al. 2007; Lejay et al. 1999; Zhuo et al. 1999), which leads to an altered root system based on nutrition. During N deficiency, the higher abundance of NRT2.1 leads to lateral root initiation, coordinating the development of lateral roots and, therefore, increases N uptake (Remans et al. 2006). An interaction with a partner protein, NAR2.1, is required for NRT2.1 to transport $NO_3^-$ (Okamoto et al. 2006; Orsel et al. 2006). Plants are also able to take up certain organic N compounds such as urea, amino acids, nucleic acids, and small peptides that are naturally present in soils or are added as fertilizers, as is the case for urea (Beier et al. 2019; Girke et al. 2014; Liu et al. 2003; Mérigout et al. 2008; Owen and Jones 2001; Sopanen et al. 1977; Waterworth and Bray 2006).

Molecular nitrogen is abundant in the atmosphere (78.1%) and is accessible to certain bacterial strains, which can fix it for their own use. As such, it presents an untapped resource that can be used for agriculture. Symbiotic biological nitrogen fixation (BNF) in nodules converts atmospheric nitrogen ($N_2$) into ammonia ($NH_3$), which is subsequently protonated to ammonium ($NH_4^+$) upon export in the peribacteroid space, which is in an acidic pH range (Day et al. 2001; Franche et al. 2009; Lam et al. 1996; Raymond et al. 2004). Free-living bacterial nitrogen fixation results in ammonia (Smercina et al. 2019), which can either be protonated in the soil to ammonium (requires acidic environment) or converted to nitrate via nitrifiers. Subsequently, second reduction of nitrate to ammonium is performed by plant metabolism, as explained above. Ammonia-oxidizing bacteria, Archea, and nitrite-oxidizing bacteria, such as *Nitrospira* bacteria, can either convert i) ammonia to nitrite and in a consecutive step to nitrate or ii) ammonia directly to nitrate via the enzymes ammonia monooxygenase or nitrite oxidoreductase, respectively (Daims et al. 2015). The ammonia used during nitrification is not necessarily derived from BNF.

One of the best-studied groups of plants that form symbiotic relationships with rhizobacteria are legumes. Readers interested in nodule-related processes can consult Gautrat et al. (2020) and Etesami and Adl (2020). Here, we will focus on non-legumes and so called free-living plant-associated microorganisms from the genus *Pseudomonas*. Historically, there are numerous examples of transfer of N from bacteria to plants in non-legume systems (Fig. 1B), however, the potential is not fully exploited yet. We propose that a greater understanding of the molecular mechanisms in the interaction between N-fixing *Pseudomonas* spp. and the plant is crucial for further development of their application in agriculture.

## Plant Growth Promotion by Bacteria of the Genus *Pseudomonas*

*Pseudomonas* spp. make up 1.6% of the bacteria in the soil and are found in all areas in the world (Trivedi et al. 2020). They attract major attention due to their various growth-promoting characteristics, good root colonization, and production of enzymes and metabolites that positively impact plant growth (Glick and Bashan 1997; Kloepper et al. 1989; Podile and Kishore 2007; Ramamoorthy et al. 2001; Saharan and Nehra 2011).

The means by which beneficial *Pseudomonas* spp. promote the growth of the host plants can be categorized into three areas (Fig. 2). The first is by producing plant growth regulators, such as gibberellin, auxins (indole acetic acid [IAA]), and cytokinin-zeatin. IAA increases seedling root growth and, thus, nutrient foraging. Often, the increase in IAA comes with a higher abundance of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which lowers the increased ethylene levels caused by stress, resulting in, e.g., an increased salt-stress tolerance (IAA, ACC-deaminase) (Egamberdieva 2009; Glick 2014; Nadeem et al. 2010). *Pseudomonas* spp. can alter salicylic acid and jasmonic acid pathways in the plant, leading to induced systemic resistance (ISR) (Glick 1995; Glick et al. 1999; Ryu et al. 2003). The second way by which *Pseudomonas* spp. can influence plants is through volatile organic compounds produced by *Pseudomonas* spp. These include geosmin, 2,3-butanediol, acetoin, and tridecane, which can promote plant growth (Panpatte et al. 2017). Other *Pseudomonas* spp. can produce compounds for biocontrol, including methanethiol, dimethyl sulfite, and dimethyl trisulfite (Panpatte et al. 2017). Finally, the third way is by *Pseudomonas* spp. increasing the availability of nutrients in the rhizosphere by nitrogen fixation, phosphate solubilization, or siderophore production, making more N, P,

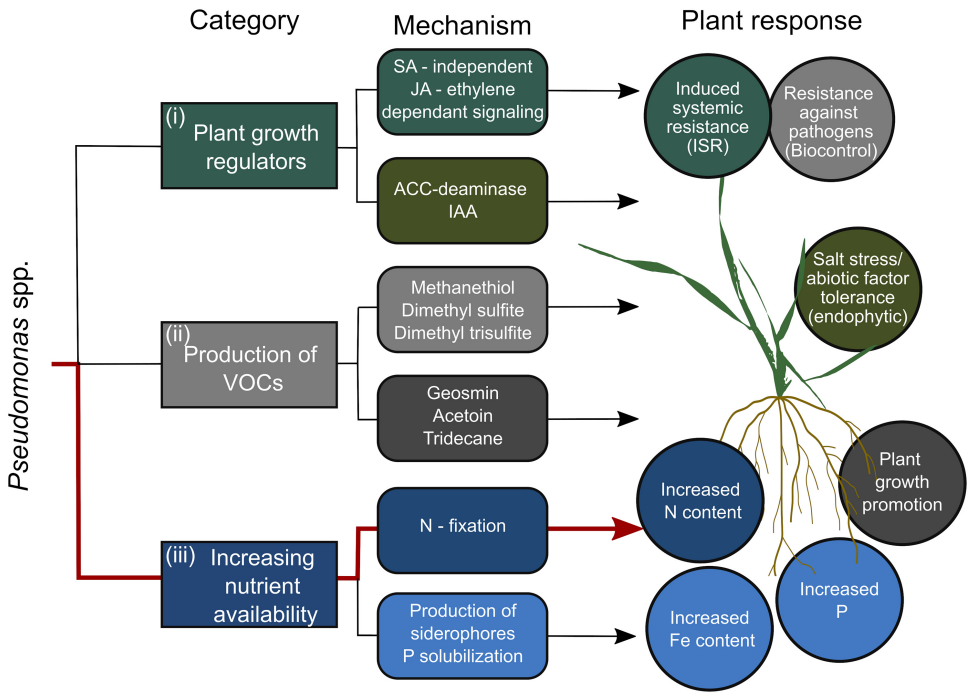


**Fig. 2.** Overview of plant growth-promoting traits by *Pseudomonas* spp. Beneficial pseudomonads can interact with plants by i) regulating plant growth via the hormonal pathways, ii) producing volatile organic compounds (VOCs), and iii) increasing nutrient availability, resulting in various plant growth-promoting traits, increased resistance against diverse pathogens and abiotic factor tolerance. SA = salicylic acid, JA = jasmonic acid, ACC = 1-aminocyclopropane-1-carboxylic acid, IAA = indole acetic acid.

and Fe, respectively, available to plants (Fig. 2) (Glick 1995; Glick et al. 1999; Kloepper et al. 1989; Liu et al. 2017; Nadeem et al. 2010; Richardson 2001; Vessey 2003; Walter et al. 1994).

## *Pseudomonas*-Driven Soil Nitrogen Biochemistry, Relevant for Improvement of Plant N

*Pseudomonas* spp. can improve the availability of inorganic N to roots by the three main strategies exemplified in Figure 3. These are i) ammonification of organic N (e.g., by *P. psychrotolerans* [Kang et al. 2020]), ii) stimulation of adjacent bacterial strains to increase N fixation (e.g., between *P. fluorescens* and *Azospirillum brasilense* [Combes-Meynet et al. 2011]), and iii) production and release of $NH_4^+$ in the rhizosphere (e.g., by *P. stutzeri* [Zhang et al. 2012]).

### Ammonification and denitrification.

The process of ammonification allows the conversion of organic nitrogen (amino acids, amino sugars, urea, nucleotides)

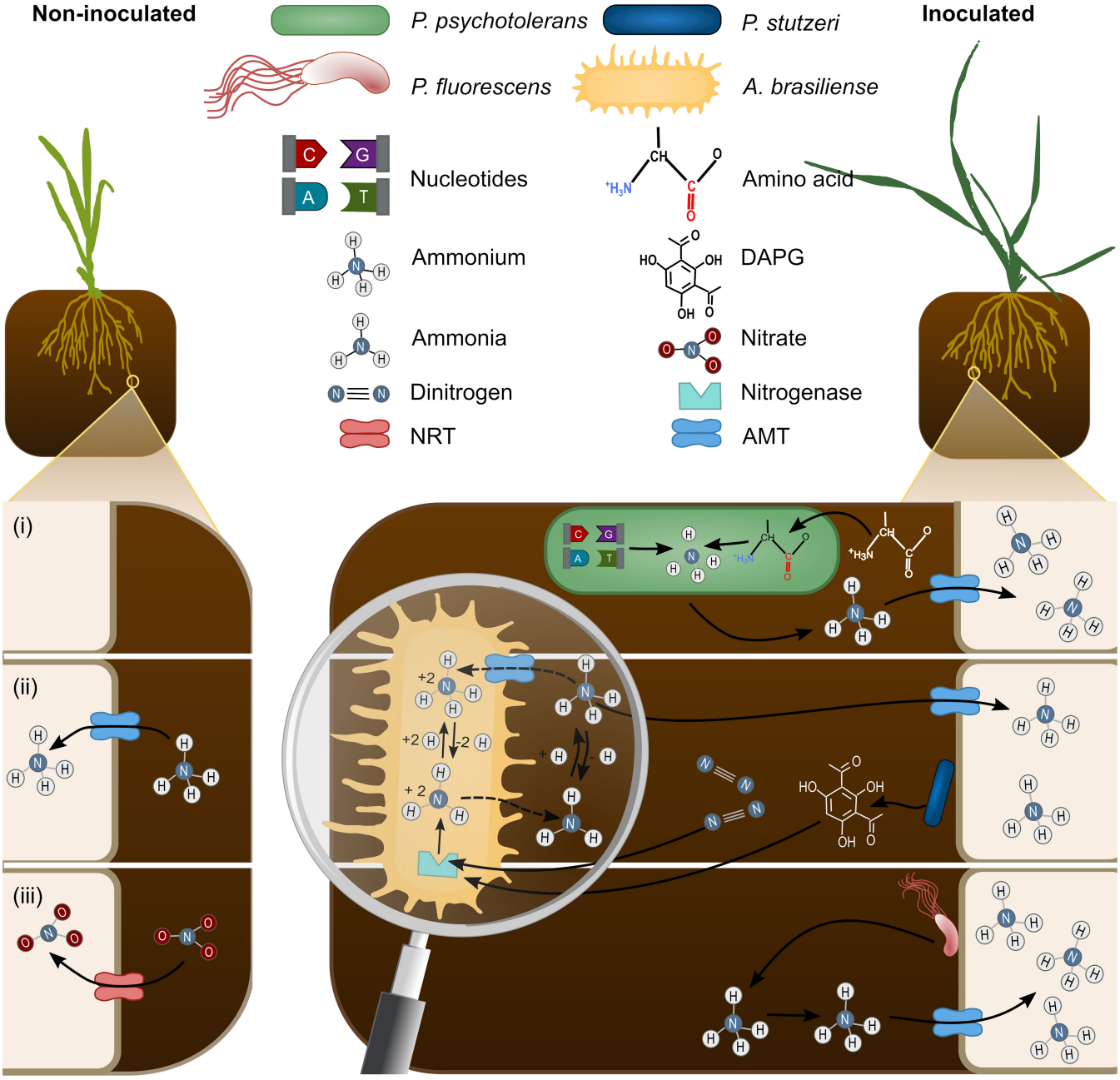


**Fig. 3.** Conceptual figure of shared nitrogen biochemistry and transport across root and bacterial cells in the rhizosphere. Bacterial processes that impact plant N content. The left side represents plants growing on limited N resulting in a decreased aerial biomass and increased root growth, whereas the right side represents potential plant growth-promoting mechanisms by *Pseudomonas* species increasing the aerial biomass under the same limited N conditions. Ammonium ($NH_4^+$) and nitrate ($NO_3^-$) are taken up by the plant via dedicated transporters of the AMT and NRT families, respectively (Bock and Wagner 2001; Daims et al. 2015). Plant growth-promoting bacteria increase availability of inorganic N to plants via following mechanisms: i) ammonification of organic N by *Pseudomonas psychrotolerans* (Kang et al. 2020), ii) *Pseudomonas stutzeri* upregulating *nif* genes in *Azospirillum brasilense* via 2,4-diacetylphloroglucinol (DAPG), resulting in the conversion of $N_2$ into $NH_4^+$ (biological nitrogen fixation) (Day et al. 2001; Combes-Meynet et al. 2011) and iii) production and release of $NH_4^+$ by *P. fluorescens* (Zhang et al. 2012). Dashed lines indicate reactions from or to the bacterium that occur based on the concentration of each reaction product in the respective space and pH of the environment.

into $NH_4^+$ (Song and Tobias 2011), which contributes to an increase of plant-available N in the soil (Figs. 3i and 4) (Kang et al. 2020). On the other hand, denitrification decreases the amount of $NO_3^-$ in the soil by reducing $NO_3^-$ to $N_2O_{(g)}$ and $N_{2(g)}$ (Fig. 4).

Until 2016, it was widely believed that denitrification and ammonification did not occur within the same bacterium. However, Torres et al. (2016) show that at least three bacteria (*Opitutus terrae* PB90-1, *Marivirga tractuosa* DSM 4126, and the gammaproteobacterium *Shewanella loihica* PV-4) contain the complete set of genes for denitrification and respiratory ammonification, based on genome analyses (Sanford et al. 2012; Torres et al. 2016). Interestingly *P. psychrotolerans* CS51 also contains genes for both processes; Kang et al. (2020) found that ammonification would be possible via the three nitrate ABC transporter genes and *NasT* (*response regulator*), while denitrification via the genes nitrite reductase (*nirB*), nitrate reductase (*napA*), and nitric oxide reductase (*norB*) (Kang et al. 2020). The enzymes nirB, napA, and norB convert nitrate to nitrite to nitric oxide and, finally, to nitrous oxide ($N_2O$), notably nosZ for the conversion to gaseous nitrogen was not found (Kang et al. 2020; Smith et al. 2007; Wang et al. 2017). Additionally, the genes involved in ammonia assimilation *GlxC* (*glutamate synthase [NADPH] putative GlxC chain* [EC 1.4.1.13]) and *GlxB* (glutamine amidotransferase protein GlxB [EC 2.4.2.-]) have been found in the genome of *P. psychrotolerans* (Kang et al. 2020). This leads to the hypothesis that *P. psychrotolerans* CS51 could also perform both ammonification and denitrification.

### Production of secondary metabolites that stimulate N fixation in adjacent bacterial strains.

In the case of *Pseudomonas fluorescens* F113 and *Azospirillum brasilense* S245, Combes-Meynet et al. (2011) found an example of positive interaction, in which one strain stimulates N fixation in another. *Pseudomonas fluorescens* F113 produces a secondary metabolite, 2,4-diacetylphloroglucinol (DAPG), that acts as a signal for *Azospirillum brasilense* S245. When *Azospirillum brasilense* detects DAPG, it upregulates the *nirK* and *nifX-nifB* genes, which leads to increased nitrogen fixation. This was demonstrated by co-inoculating wheat with both strains (Combes-Meynet et al. 2011) (Figs. 3ii and 4; Table 1). Sequence analysis of DAPG-induced promotors, followed by functional prediction of the corresponding downstream open reading frame, revealed that their deduced protein sequences are homologous to the nitrogen-fixation gene *nifX-nifB* (Combes-Meynet et al. 2011; Rubio and Ludden 2008). This highlights the importance of using bacterial combinations (communities) in which the members function in an additive manner to unlock their full potential.

However, it needs to be kept in mind that using bacterial inoculants in the field will lead to interactions with the native microbiome. While the full extent of the interactions is not clear, we speculate that there might be events in which inoculants may become overrun by the native microbial varieties or, like in the case of *P. taiwanensis* used for maize inoculation and *P. fluorescens* LBUM677 and oilseed crops, there might be changes in the microbiome around the plant roots (Chaudhary et al. 2021; Jiménez et al. 2020). Further understanding of the suitability of inoculants to specific soil environments is needed to ensure reproductive results in the field.

### Production and release of $NH_4^+$ via BNF in *Pseudomonas* spp.

Another step of enhancing the N available for plants is achieved by ammonium excretion of bacteria (Fig. 3iii). The step of ammonification, oxidation of organic N to ammonium, releases energy for metabolic processes. The excess ammonium will be excreted by the microorganism to avoid ammonium toxicity and can either be used for nitrification or taken up by the plant via dedicated AMT transporters (if excreted by root-associated bacteria). The *Pseudomonas* genes *amtB1* and *amtB2* have been demonstrated to regulate the internal ammonia pool and excretion of ammonium (Zhang et al. 2012) (Table 1). A *P. stutzeri* A1501 mutant expressing *nifA* constitutively showed an increased ammonia excretion. Coincidently, no increase in nitrogenase activity was detected, leaving the role of constitutively expressed *nifA* unclear (Zhang et al. 2012).

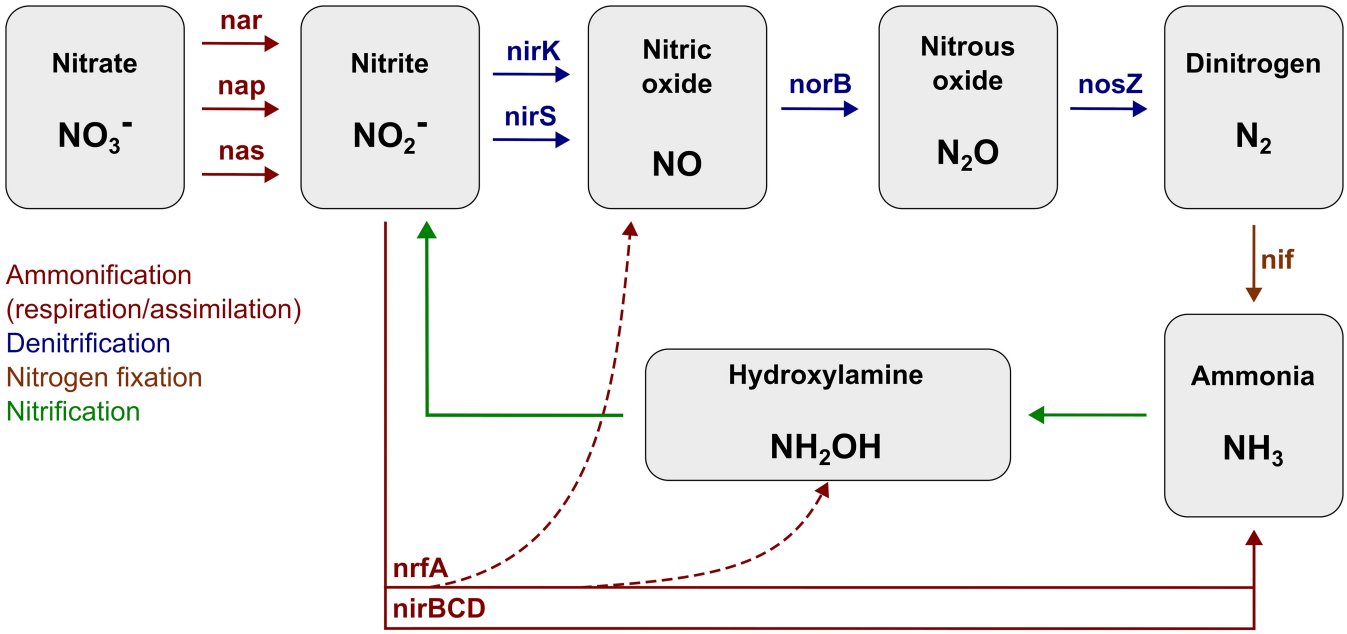


**Fig. 4.** Bacterial inorganic nitrogen cycle. Ammonification, denitrification, nitrogen fixation, and nitrification are displayed by colored solid lines, with genes involved in the pathway. Dotted lines show additional formation of nitric oxide and hydroxylamine during nitrite ammonification. Figure adapted from Rodionov et al. 2005.

## *Pseudomonas* Genetic Machinery That Could Provide Alternative Nitrogen

Diazotrophic bacteria fix nitrogen via proteins encoded by the nitrogenase gene cluster, and the organisation, abundance and regulation of nitrogen-fixation related genes is different from species to species (Yan et al. 2008). Several species in the family of Pseudomonaceae, including *Azotobacter vinelandii* AvOP, *Pseudomonas stutzeri* A1501, *Pseudomonas stutzeri* DSM4166, *Pseudomonas szotifigens* 6HT33bT and *Pseudomonas* sp. strain K1 have been demonstrated to be capable of nitrogen fixation (Setten et al. 2013).

Specific to *Pseudomonas*, a nitrogen-fixation island (NFI) was discovered and found to be conserved in *P. stutzeri* strains from geographically close (<300 km within Greece) or distant (China, Germany, Greece) locations (Venieraki et al. 2014). The NFI was presumably acquired by lateral gene transfer of a common ancestor of *Klebsiella pneumoniae*, *Pseudomonas stutzeri* and *Azotobacter vinelandii* and inserted between cobalamin synthase (*cobS*) and glutathione peroxidase (*gspH*) (Yan et al. 2008, Setten et al. 2013).

The size of the NFI in *P. stutzeri* is about 49 kb and contains 52 *nif*-related genes, which are organized into 11 putative NifA-σ54-dependent operons (Table 1, Yan et al. 2010; Yang et al. 2018). The σ54 transcription factor (or RpoN) is a well-known alternative factor for RNA polymerase that enables the transcription of *nif* genes in conjunction with the transcriptional activator NifA or activates other genes involved in nitrogen metabolism and functions with the nitrogen regulatory protein C (NtrC), the *psp* operon transcriptional activator (*pspF*), and C4-dicarboxylate transport transcriptional regulatory protein DctD (Yan et al. 2010).

*Pseudomonas protegens* Pf-5 was genetically modified to contain the X940 cosmid, which includes all genes (numbered from PST1302 to PST1359) from the nitrogen fixation island of *P. stutzeri* A1501. X490 with nitrogenase activity was transferred to various other *Pseudomonas* spp., including *P. putida*, *P. veronii*, *P. taetrolens*, *P. balearica*, and *P. stutzeri* (Setten et al. 2013). A constitutive nitrogenase activity and high ammonium production was observed in all mentioned strains except *P. balearica* and *P. stutzeri*, indicating that a genome context is required for the activity of the X940 cosmid (Setten et al. 2013).

Nitrogen fixation and assimilation are intrinsically coupled and controlled by complex regulatory circuits, favoring the assimilation of fixed nitrogen into biomass instead of excreting it. The processes are controlled by the PII complex, which includes signal transduction proteins such as GlnB and GlnK, enzymes responding to metabolite control via reversible post-translational modifications of proteins (GlnD and GlnE), and a two-component regulatory system (NtrB or NRII and NtrC or NRI) controlling many nitrogen metabolism genes. PII tightly regulates NifA, a positive regulatory element (Table 1) to fix nitrogen by demand. Readers wanting a comprehensive review can refer to Bueno Batista and Dixon (2019).

To unravel the underlying genetic mechanism of the *P. stutzeri* NFI genes, the complete NFI was transferred into the recombinant *Escherichia coli* EN-01 and was investigated by transcriptomics and proteomics for functional investigation (Yang et al. 2018). In the genetically engineered *E. coli* EN-01 harboring the heterologous NFI island from *P. stutzeri*, primary nitrogen assimilation is achieved by synthesis of glutamate from ammonium and 2-oxoglutarate, catalyzed by two alternative pathways (Yang et al. 2018). In an N-rich environment, the pathway involving the enzyme glutamate dehydrogenase was active. Alternatively, in an N-limited environment, the enzymes glutamate synthetase (GS [gene *glnA*]) and glutamine oxoglutarate aminotransferase (GOGAT [gene *gltBD*]) were predominantly active (Yang et al. 2018). This is in agreement with data showing that bacterial cel-

**Table 1.** The *nif* genes and their known or proposed roles during nitrogen fixation in all species[a]

| Cluster/gene | Function |
|---|---|
| *nifQ* | Involved in FeMo-co synthesis; proposed to function in early $MoO_4^{2-}$ processing |
| *nifB* | Required for FeMo-co synthesis; its metabolic product, nifB-co, is a specific Fe and S donor to FeMo-co |
| *nifA* | Positive regulatory element |
| *nifL* | Negative regulatory element |
| rnfABCDGEH | Involved in electron transport to nitrogenase, possibly functioning to drive the thermodynamically unfavorable reverse electron transfer from NADH to ferredoxin |
| nifHDKTY | |
| *nifH* | Fe protein; required for FeMo-co biosynthesis and apo-MoFe protein maturation; electron donor to MoFe protein during nitrogenase |
| *nifD* | Alpha subunit of MoFe protein; forms an $\alpha_2\beta_2$ tetramer with the β subunit; the site of substrate reduction, FeMo-co, is within the α subunit of MoFe protein |
| *nifK* | Beta subunits of MoFe protein P clusters are present at each αβ subunit interface |
| *nifT* | Unknown |
| *nifY/nafY* | Chaperone for the apo-MoFe protein NafY is also a FeMo-co carrier and is proposed to aid in the insertion of FeMo-co into apo-MoFe protein |
| nifENX | |
| *nifE* | Forms $\alpha_2\beta_2$ tetramer with NifN; required for FeMo-co synthesis; proposed to function as a scaffold on which FeMo-co is synthesized |
| *nifN* | Required for FeMo-co synthesis; tetramer with NifE |
| *nifX* | Involved in FeMo-co synthesis; accumulates a FeSMo-containing precursor |
| nifUSV | |
| *nifU* | Molecular scaffold for the formation of Fe-S cluster for nitrogenase components |
| *nifS* | Involved in mobilization of S for Fe-S cluster synthesis and repair |
| *nifV* | Homocitrate synthase, involved in FeMo-co synthesis |
| nifWZM | |
| *nifW* | Involved in stability of MoFe protein |
| *nifZ* | Unknown |
| *nifM* | Required for the maturation of NifH |
| *nifF* | Flavodoxin; physiological electron donor to NifH in *Klebsiella pneumoniae* |
| modABC | Encodes an ABC-type, high-affinity, molybdate transporter |
| *hesB* | Binding to a 2 iron, 2 sulfur (2Fe-2S) cluster; this cluster consists of two iron atoms, with two inorganic sulfur atoms found between the irons and acting as bridging ligands |
| *cysE* | Biosynthesis of cysteine |

[a] As reported by Rubio and Ludden 2005; Yan et al. 2008.

lular nitrogen is composed of 75 to 88% of glutamate and 12 to 25% glutamine, which act as main nitrogen donors (Prusiner and Stadtman 1973; Yang et al. 2018).

In *P. stutzeri* A1501, the expression of the nitrogen fixation–specific regulatory protein NifLA is controlled by the nitrogen regulatory cascade AmtB-GlnK-NtrBC (Yang et al. 2018). During N-fixing conditions, genes of the NFI had a more than tenfold higher transcript level and were downregulated after ammonium shock (Yan et al. 2010; Yang et al. 2018). *NtrC* and *glnK* are necessary for the expression of NifA, and in turn NifA is expected to play a major role in the expression of *Ntr* (nitroreductase) genes. Under constitutive expression of NifA in a *P. stutzeri* A1501 *glnK*$^{-}$ mutant, nitrogenase activity and nitrogen fixation have been restored and observed under presence of ammonia (He et al. 2008). Thus, it was proposed that GlnK acts as a key regulatory element in control of ammonia assimilation, *nifA* expression, and in modulation of NifA activity by NifL (He et al. 2008).

The study by Yang et al. 2018 showed that other microbes can be genetically engineered to acquire the ability to fix nitrogen via the NFI. Furthermore, it successfully combines multi-omics approaches in the study of microbes and their metabolic processes.

Understanding the microbial process of N fixation is fundamental to shed light on the molecular mechanism of plant-microbe interactions (PMI). However, this knowledge must be put into context with plant responses also, to fully understand the interaction between plants and *Pseudomonas* spp. during N fixation.

## Plant Molecular Components Involved in Uptake of *Pseudomonas*-Derived N

### Root nitrogen transporters and central N metabolism.

It has been shown that, in cases of excess or deficient N supply, the plant transporters of the AMT and the nitrate transport system (NRT) will be adjusted accordingly, together with other crucial enzymes of central N metabolism, such as nitrate reductase, nitrite reductase, GS, and GOGAT (Muratore et al. 2021).

To check if this is true under bacterial inoculation, Trinh et al. (2018) investigated the expression of N-related genes during *A. thaliana* and *Lactuca sativa* (lettuce) interaction with *P. nitroreducens* IHB B 13561. They focused on the nitrate transporter genes *NRT1.1*, *NRT1.2*, *NRT2.1*, *NRT2.2*, *NRT2.5*, and *NRT2.6* (Mantelin et al. 2006); *NIR1*, *NLP6*, and *NLP7*, which are involved in nitrate response; *EIN1* and *ERF1*, which play roles in the ethylene response pathway; *ARF19*, *ARF7*, and *AXR4*, which are involved in the auxin signaling pathways; and the ammonium transporters *AMT1.1*, *AMT1.2*, and *AMT2* (Trinh et al. 2018).

Out of these genes in *A. thaliana*, higher expression levels were found for *NRT2.1*, *NRT2.2*, and *NRT2.6*.

However, a lower N content was measured in the seedlings during inoculation with *P. nitroreducens* IHB, which the authors assumed to be based on a higher rate of cell division requiring a more rapid nitrate metabolism. In return, a lower inner nitrate content induced NRT2 genes, increasing nitrate uptake (Trinh et al. 2018). Thus, in *Arabidopsis*, *P. nitroreducens* IHB influenced root physiology (Fig. 5).

Similarly, in lettuce, three nitrate transporter genes (*LsNRT1*, *LsNRT2*, *LsNRT2.5*) were examined after inoculation with *P. nitroreducens* IHB. *LsNRT1* and *LsNRT2* showed higher levels of expression. LsNRT2 and AtNRT2.1 are highly similar in their sequence alignments, suggesting similar responses in these two different species. Consequently, *P. nitroreducens* IHB inoculation increased the nitrate uptake and promoted growth by increasing the levels of transcripts of NRT2.1 in both *A. thaliana* and *L. sativa* (Fig. 5) (Poitout et al. 2017; Trinh et al. 2018). In addition, metabolic stimulation and induction of cell development is proposed to be a driving force for the growth promotion in both *A. thaliana* and *L. sativa* by *P. nitroreducens* IHB (Trinh et al. 2018). It is speculated that ammonium is primarily used for the elevated cell division to synthesize essential proteins and other compounds (Howitt and Udvardi 2000; Pratelli and Pilot 2014). Interestingly, increased transcripts of transporters of the NRT2 family were observed in both *L. sativa* and *A. thaliana* during this study, while the nitrate levels in the plant declined (Trinh et al. 2018), indicating that the assimilation of nitrate is preferred over storage in the vacuole. *P. nitroreducens* IHB seems to be a promising candidate for improved plant performance by increasing plant growth in *A. thaliana* and *L. sativa* and improving soil nitrate utilization (Fig. 5, top) (Trinh et al. 2018).

*Solanum lycopersicum* (tomato) inoculated with *P. fluorescens* Pf-16 showed a molecular reprogramming during the early stages of inoculation (Fig. 5, middle) (Scotti et al. 2019). The stages can be distinguished into <48 h and >48 h. During the earlier stage, Pf-16 inoculation led to increased gene expression of *AMT1.3* (Solyc03g045070.1.1) while decreasing gene expression of *NRT2.1* (Solyc00g090860.1.1) and *NRT2.4* (Solyc11g069760.1.1) (Fig. 6). In the later stage, almost no changes in the expression of *AMT1.3*, *NRT2.1*, and *NRT2.4* were detectable, while cell-wall modifications and upregulations of plant growth-promotion genes were observed (Scotti et al. 2019). The main finding of the study by Scotti et al. (2019) was that Pf-16 inoculation can both increase the tolerance against biotic and abiotic stresses and promote plant growth simultaneously. However, the authors have also shown that nitrogen-related transcript dynamics during the early stage of PMI, measuring the plant response over a longer period, might give further insight in the molecular mechanism. Those dynamics could be used to identify the timepoint of growth promotion during PMI and would clarify whether the plant responds only for a short period after inoculation, requiring more frequent inoculations to improve plant growth, or if a single inoculation is sufficient.

### Amino acid metabolism.

Besides changes on N-related transporters or enzymes, other pathways are involved in promoting plant growth during PMI. Transcription analyses discovered the upregulation of two genes in *Arabidopsis* inoculated with *P. putida* MTCC5279, namely, At3g47340, a glutamine-dependent asparagine synthase 1, and At3g10340, an ammonia lyase (Fig. 5, bottom) (Srivastava et al. 2012). Asparagine, due to its high N:C ratio and stability, is the preferred form for long-distance transport and storage of N in most higher plants and can account for 86% of transported N (Miflin and Lea 1980; Urquhart and Joy 1981; Sieciechowicz et al. 1988). The higher abundance of transcripts of the glutamine-dependent asparagine synthase 1 (At3g47340) indicate that assimilated nitrogen might be converted into glutamine and, further, into asparagine for potential transport within the plant during PMI. Thus, it might be interesting to analyze the amino acid profile of plants during PMI to investigate this hypothesis. At3g10340 encodes phenylalanine ammonia lyase 4 (PAL4), which is involved in the phenylpropanoid pathway, catalyzing the first step of non-oxidative deamination of L-phenylalanine to 7,8-unsaturated *trans*-cinnamic acid and an ammonium ion. Ammonium is directly recycled via the GS/GOGAT pathway to regenerate arogenate, which is required for further metabolism of cinnamic acid to various phenylpropanoids and their derivatives. The phenylpropanoid pathway is responsible for the synthesis of a large range of natural products in plants, including flavonoids (pigments and UV protectants), the structural polymer lignin, and antimicrobial furanocoumarin and isoflavonoid phytoalexins (Hahlbrock and

Scheel 1989; Dixon and Paiva 1995). Salicylic acid, which is involved in the establishment of both local and systemic plant defense responses, is also a product of this pathway (Klessig and Malamy 1994).

**Open questions.**

Despite several approaches to study molecular mechanisms, a big challenge is missing characterizations of proteins involved in PMI, showing the importance of functional characterization approaches to update databases and allow further research in this area. One example is a study about the interaction of *P. fluorescens* PICF7 with wheat and barley on a molecular basis by performing a proteomics approach (Fröhlich et al. 2012). The root proteome of inoculated seedlings of wheat and barley revealed 14 and 24 proteins exclusively abundant during PICF7 inoculation, respectively. However, only three of the 14 proteins in wheat have shown similarities to databases, namely a putative Nodal modulator 3, a phosphoenolpyruvate carboxylase, and an *S*-formylglutathione hydrolase-like protein, whereas the proteins of barley are predicted or uncharacterized proteins (0 of 6 known proteins in barley).

## BNF in *Pseudomonas* spp. and the Influence of the Abiotic Environment to Plant Growth Promotion

To test the contribution of BNF to total plant N content, maize and wheat were grown in $^{15}$N-supplemented soil, with *P. prote-*

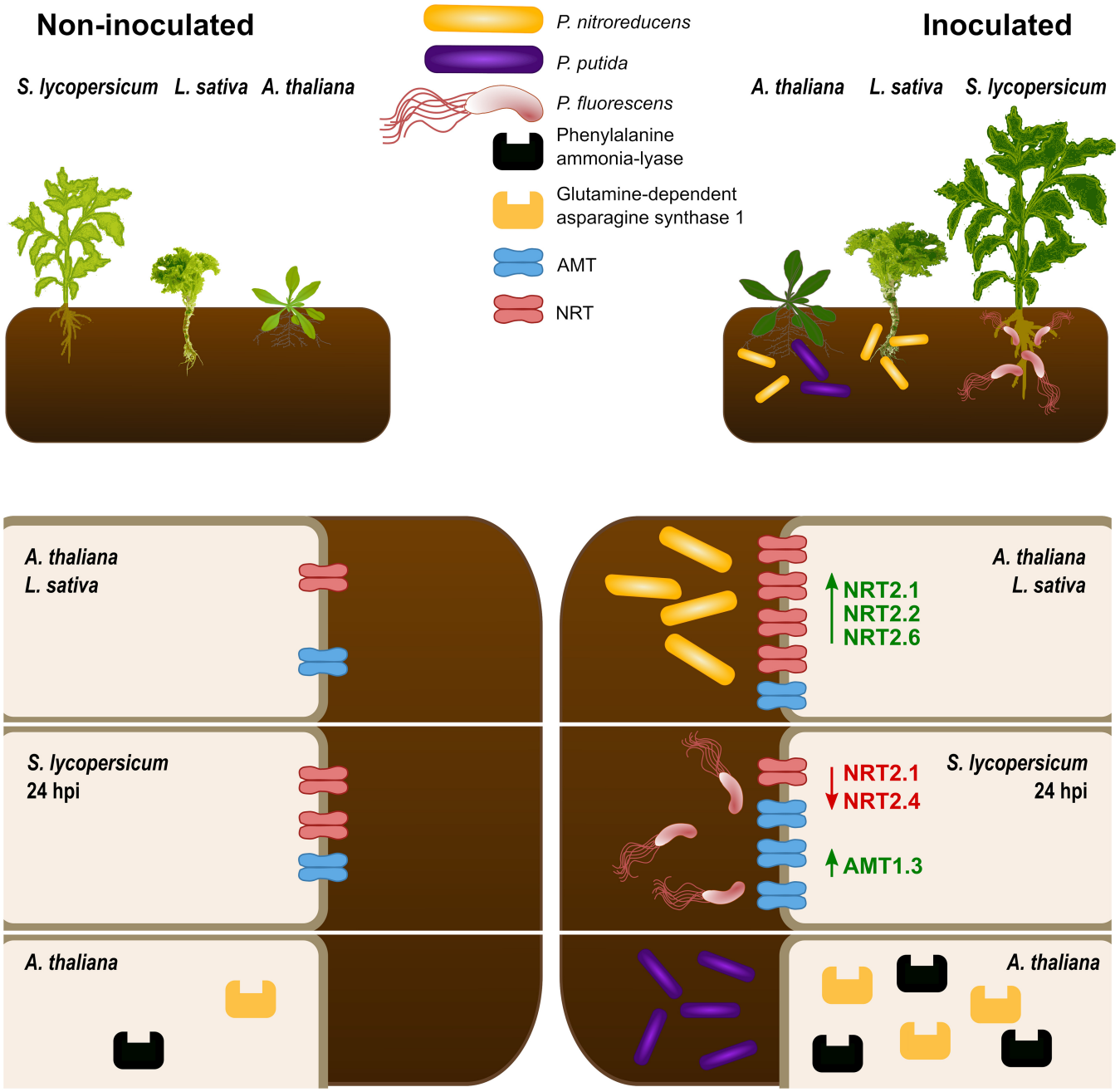


**Fig. 5.** Molecular mechanisms of plant-microbe interactions. Colonization with beneficial bacteria alters the expression of genes, transcripts, and proteins. Inoculation with *Pseudomonas nitroreducens* increases the abundance of N-related transporters NRT2.1, NRT2.2, NRT2.6, while inoculation with *Pseudomonas fluorescens* increases abundance of AMT1.3 but decreases NRT2.1 and NRT2.4. Genes encoding glutamine-dependent asparagine synthase 1 and phenylalanine ammonia lyase are upregulated in response to inoculation with *Pseudomonas putida*.

*gens* Pf-5 with and without the X940 cosmid in the greenhouse (Table 2) (Fox et al. 2016). $^{15}N$ was used to increase the $^{15}N/^{14}N$ ratio in the soil. Thus, nitrogen originating from the air would have a comparatively higher $^{14}N$ fraction than that from soil and would be indicative of N fixation. BNF was proposed to be active from the early stages of plant growth, since measurements of the $\delta^{15}N$ value 1 month after inoculation showed significantly lower $^{15}N$ in root, leaf, and stem tissues of Pf-5 X940-treated maize and wheat plants than in those of Pf-5-treated plants (Fox et al. 2016). Two months after inoculation, the nitrogen content derived from gaseous nitrogen (%Ndfa) in Pf-5 X940-treated maize and wheat plant organs was 74 and 85% for roots, 63 and 78% for leaves, and 70 and 82% for stems, respectively, indicating the assimilation of nitrogen derived from air. Plant growth promotion was also shown in various flowering plant species inoculated with Pf-5 X940 under sterile hydroponic growth-chamber conditions (Table 2). The beneficial effect might be the result of an increased ammonium excretion (Fox et al. 2016).

Confocal microscopy with green fluorescent protein–tagged Pf-5 and Pf-5 X940 demonstrated that both strains appear to colonize solely the rhizosphere and neither the endosphere nor the phyllosphere (Fox et al. 2016). Due to the functionality of the X940 cosmid in different *Pseudomonas* spp., transformation of established *Pseudomonas* strains with the X940 cosmid and application in agriculture might lead to increased plant performance and yield.

Ke et al. (2019) tested the contribution of *P. stutzeri* A1501 to BNF in well-watered and water-deficient conditions using maize. Using sterilized soil, an increase of 27.8 %Ndfa was measured in well-watered conditions, while under water deficiency only 17.5 %Ndfa was observed. In absolute values, a total of 0.90 g of plant N was fixed in well-watered conditions and 0.30 g of plant in water-deficient conditions. When grown in natural soil, the nitrogen derived from $N_2$ was 0.72 and 0.18 g per plant (23.6 and 14.1%) for well-watered and water-deficient conditions, respectively. Ke et al. (2019) was able to show the actual amounts of nitrogen fixed by *P. stutzeri* A1501, both in sterile and natural conditions. This shows the potential use of *P. stutzeri* A1501 to increase total N content in plants and therefore increase the biomass production (Ke et al. 2019) and underlines the importance of water supply for successful PMI.

*P. psychrotolerans* CS51, having genes for both denitrification and ammonification, could not only be used for plant growth promotion but also for denitrification of soils. This could mean that, in anoxic environments, such as soils containing a lot of water (like rice fields), $NO_3^-$ could be used for denitrification, while in dry soils, respiratory ammonification occurs (Torres et al. 2016). As denitrification is not linked to plant growth promotion, the application of this strain seems to be limited to plants growing in dry soils. This strain might become more relevant with the future challenges we are facing, such as climate change and drought. Additionally, it indicates that soil properties impact the direction of the chemical reaction. Importantly, increased metabolism involving $NO_3^-$ could remove highly abundant nitrate from the soil, resulting in a decreased nitrate leaching to ground water.

Afzal et al. (2010) shows that not only the water and nitrogen availability or $N_2$-fixation play a major role in increasing the plant N content but that other nutrients are important too. The authors tested the co-inoculation of *Glycine max* (soybean) with *Bradyrhizobium japonicum* TAL 377 and *Pseudomonas* sp.

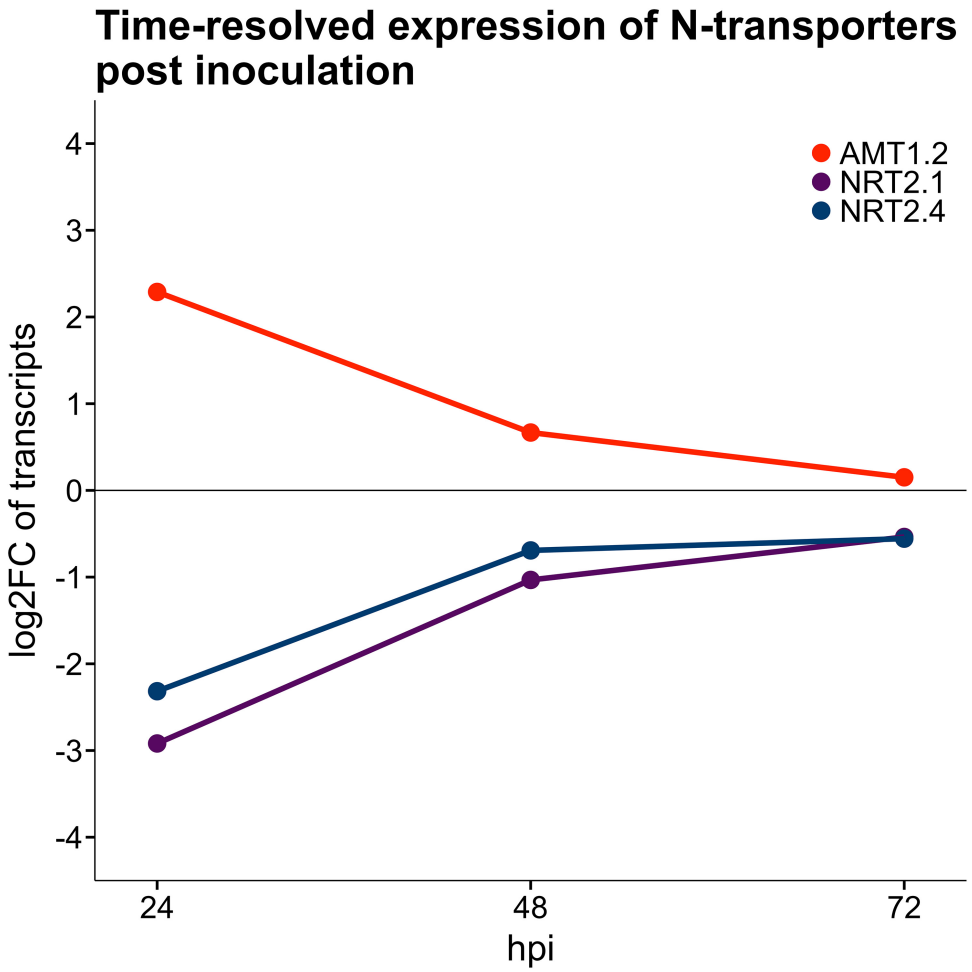


**Fig. 6.** Time-resolved changes on transcript level of N-related transporters postinoculation with *Pseudomonas fluorescens*. $Log_2$ fold changes (log2FC) of AMT1.3, NRT2.1, NRT2.4 in *Solanum lycopersicum* roots inoculated with *P. fluorescens* 24, 48, and 72 h postinoculation (hpi). A strong early response can be observed soon after inoculation (24 hpi), which declines over time (72 hpi). During the early stage of inoculation (24 hpi), NRT2.1 and NRT2.4 are downregulated, whereas AMT1.3 is upregulated. Data from Scotti et al. (2019).

strain 54RB under varying phosphorus availability. For the pot experiments, a dose of 0.3 g of nitrogen per pot was applied in the form of urea, and different phosphorus levels were introduced by adding either no additional P or 1.35 g of $P_2O_5$ (50 kg per hectare) in the form of single superphosphate (SSP) to treated conditions. However, one must keep in mind that SSP also contains Ca and S, indicating that the following plant phenotypic changes are not only based on increased P but also increased Ca and S levels. Co-inoculation with both strains had positive effects in $P_2O_5$-treated conditions in terms of number of pods, grain yield, seed N and P, and total available P and N in soil. The available N content in soil was increased by 72% over $P_2O_5$-only treated plants, indicating that the availability of other nutrients (nutrient crosstalk) is also decisive for better N content in plants (Afzal et al. 2010). Observing *Pseudomonas* sp. strain 54RB promoting plant growth on its own and increasing available N in cooperation with another strain hints to its compatibility in microbial communities, and future combinations could include P-solubilizing microbes to avoid limitations, as per Liebig's law of the minimum. The exchange of nutrients and carbon between plants and microbes can occur at the plant root surface or within the plant root tissue (symplastic, apoplastic). The cumulative activity of the bacterial transmembrane transporters, the so-called transportome, plays a crucial role in PMI and determines which ecological niche is occupied in the rhizosphere (Silby et al. 2009; Larsen et al. 2015; Wilton et al. 2018). The activity of the bacterial transportome of several *Pseudomonas* spp. can lead to protection against nutrient stresses (Shinde et al. 2017; Wilton et al. 2018).

Moreover, the ability of many *Pseudomonas* spp. to grow at lower temperatures ensures propagation and survivability in fields, despite changing temperatures of the soils (Kwon et al. 2003). An outstanding example is *Pseudomonas migulae*. *P. migulae* was isolated from the Chhiplakot region, in the Western Indian Himalaya (Suyal et al. 2014). This $N_2$-fixing strain has adapted to different abiotic stresses, including cold temperature, height, and oxidative stress, which made it possible to fix N at temperatures of 5 to 10°C (Suyal et al. 2014). This shows once more that testing different strains from various geographical locations is valuable, as it gives insight into the mechanistic acclimation to varying environmental factors (*P. stutzeri* tested from different geographical locations [Venieraki et al. 2014]). Consequently, beneficial *Pseudomonas* species could be collected, applicable for fields in various geo-climatic regions.

## Conclusions and Open Questions

A number of plant species were tested with *Pseudomonas* spp. and have shown beneficial responses that can be linked to improved N content in the plant. This encourages the further study of the use of *Pseudomonas* spp. for future agricultural applications, to ultimately reduce the application of N fertilizer while maintaining the required amount of biomass. Due to the ubiquitous distribution of the genus in the world soils and, inherently, to the adaptation to most global conditions, strains can be found that will work in fields across the globe. Despite the potential depicted, we here note that many of the mentioned studies tested the plant growth-promoting effects in natural soil but not directly in the field. Field trials come with additional challenges, like ever-changing conditions and microbial communities, compared with controlled environments. Nevertheless, *P. stutzeri* A1501 stands out as a good candidate for plant growth promotion in conditions with limited water supply (Ke et al. 2019). One additional gap is clear understanding of how these strains would perform in field microbial communities and under a variety of abiotic stresses.

In terms of yield, no clear evidence is displayed in this review, leaving the question open. Furthermore, we must keep in mind that, although we focus on N-fixation, *Pseudomonas* spp. (as other strains) have the potential to promote plant growth by promoting a greater root system, which in turn will reflect in better nutrient scavenging. An important example for this was recently

**Table 2.** Summary of plant phenotype responses upon inoculation with various *Pseudomonas* spp.[a]

| Plant species | Inoculant | Sterile | Plant response (inoculated vs. control) | | | Citation |
|---|---|---|---|---|---|---|
| | | | Nutrient content | Biomass[a] | Root phenotype | |
| *Zea mays* (Pannar BIOGENE BG6607YR) | PF-X940 | X | Shoot: +170% (N)<br>Seed: +556% (N) | | Bacteria contribution to N: increased | Fox et al. 2016 |
| *Triticum aestivum* (Bobwhite 26) | PF-X940 | X | Shoot: +85% (N)<br>Seed: +379% (N) | | Bacteria contribution to N: increased | Fox et al. 2016 |
| Maize | *P. stutzeri* A1501 | X | Shoot: +14.1–23.6% (N) | SDW: +20.2%<br>RDW: +31.2% | | Ke et al. 2019 |
| | *P. stutzeri* A1501 (natural growth conditions) | √ | Shoot: +17.5–27.8% (N) | SDW: +59%<br>RDW: +93% | | Ke et al. 2019 |
| Rice (Super Basmati) | *Pseudomonas* sp. strain K1 | X | | SDW: +100%<br>Seed: +55% | | Mirza et al. 2006 |
| Rice (Super Basmati 385) | *Pseudomonas* sp. strain K1 | X | | SDW: +59%<br>Seed: +93% | | Mirza et al. 2006 |
| *Brassica juncea* (brown mustard) | *P. aeruginosa* (seed-coated) | X | Whole-plant: +40.61% (N); +100% (P) | | Root length: +25% | Roychowdhury et al. 2019 |
| | *P. aeruginosa* (rhizosphere-inoculation) | X | Whole plant: +19.9% (N) | | Root length: +6.25% | Roychowdhury et al. 2019 |
| *Phaseolus vulgaris* L. (common bean) | *Rhizobium* sp. + *Pseudomonas* sp. strain Luc2 | X | Shoot: −8% (N)<br>Bacteria contribution to N: −31.4% (N) | SDW: −1.25%<br>RDW: +5.76% | Root length: +21% | Stajkovic et al. 2011 |
| *Phaseolus vulgaris* L. (common bean) | *Rhizobium* sp. + *Pseudomonas* sp. strain LG | X | Shoot: +29.1% (N)<br>Bacteria contribution to N: +113.8% (N) | SDW: +27.9%<br>Root: −2.2% | Root length: +13.4% | Stajkovic et al. 2011 |
| *Glycine max* (soybean) | *Pseudomonas* sp. strain 54RB | X | Seed: +17.4% (N); +11.9% (P) | Seed: +75.6%<br>Shoot: +29.5%<br>Root: +21.7% | | Afzal et al. 2010 |

[a] SDW = shoot dry weight; RDW = root dry weight.

published for a different clade of bacteria, certain *Oxalobacteraceae* spp., particularly of the genus *Massilia*, that affect lateral root development of maize and thereby increase N acquisition under N-limitation without bacterial $N_2$ fixation (Yu et al. 2021). Interestingly, only certain maize genotypes were able to attract and stimulate the propagation of these bacteria by secreting distinct flavones under N limitation, suggesting that crops can influence the abundance of root-associated microbiota via exudation of specific compounds (Yu et al. 2021). This, in consequence, suggests that root-rhizobacteria interactions cannot only be established by inoculation of plants with beneficial microbes but, potentially, more robustly, by targeted plant breeding, which we propose should be investigated for crops other than maize.

We note a gap in understanding the plant responses on the protein level and through plant development (e.g., from moment of inoculation) as this was only addressed in a few studies. In further approaches, key regulatory genes, peptides, proteins, enzymes, signaling molecules, and metabolites and lipids could be investigated and used for testing potential conserved plant growth–promoting domains throughout the *Pseudomonas* genus. In the ideal case, this knowledge could be transferred to other genera of bacteria.

## Literature Cited


Afzal, A., Bano, A., and Fatima, M. 2010. Higher soybean yield by inoculation with N-fixing and P-solubilizing bacteria. Agron. Sustain. Dev. 30:487-495.

Bakkou, N. 2011. Characterization of the endosymbiotic forms of *Sinorhizobium* sp. strain NGR234. Ph.D. dissertation. University of Geneva, Geneva, Switzerland.

Beier, M. P., Fujita, T., Sasaki, K., Kanno, K., Ohashi, M., Tamura, W., Konishi, N., Saito, M., Imagawa, F., Ishiyama, K., Miyao, A., Yamaya, T., and Kojima, S. 2019. The urea transporter DUR3 contributes to rice production under nitrogen-deficient and field conditions. Physiol. Plant. 167:75-89.

Ben Hassen, T., and El Bilali, H. 2022. Impacts of the Russia-Ukraine war on global food security: Towards more sustainable and resilient food systems? Foods 11:2301.

Bindel, N., and Neuhäuser, B. 2021. High-affinity ammonium transport by *Arabidopsis thaliana* AMT1;4. Acta Physiol. Plant. 43:1-5.

Bock, E., and Wagner, M. 2001. Oxidation of inorganic nitrogen compounds as an energy source. Pages 457-495 in The Prokaryotes: A Handbook on the Biology of Bacteria, M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt, eds. Springer, New York.

Boddey, R. M., De Oliveira, O. C., Urquiaga, S., Reis, V. M., De Olivares, F. L., Baldani, V. L. D., and Döbereiner, J. 1995. Biological nitrogen fixation associated with sugar cane and rice: contributions and prospects for improvement. Pages 195-209 in: Management of Biological Nitrogen Fixation for the Development of More Productive and Sustainable Agricultural Systems: Extended Versions of Papers Presented at the Symposium on Biological Nitrogen Fixation for Sustainable Agriculture at the 15th Congress of Soil Science, Acapulco, Mexico, 1994 Springer, Dordrecht, The Netherlands.

Bueno Batista, M., and Dixon, R. 2019. Manipulating nitrogen regulation in diazotrophic bacteria for agronomic benefit. Biochem. Soc. Trans. 47:603-614.

Cerezo, M., Tillard, P., Filleur, S., Muños, S., Daniel-Vedele, F., and Gojon, A. 2001. Major alterations of the regulation of root $NO_3^-$ uptake are associated with the mutation of *Nrt2.1* and *Nrt2.2* genes in Arabidopsis. Plant Physiol. 127:262-271.

Chaudhary, P., Khati, P., Chaudhary, A., Maithani, D., Kumar, G., and Sharma, A. 2021. Cultivable and metagenomic approach to study the combined impact of nanogypsum and *Pseudomonas taiwanensis* on maize plant health and its rhizospheric microbiome. PLoS One 16: e0250574.

Combes-Meynet, E., Pothier, J. F., Moënne-Loccoz, Y., and Prigent-Combaret, C. 2011. The *Pseudomonas* secondary metabolite 2,4-diacetylphloroglucinol is a signal inducing rhizoplane expression of *Azospirillum* genes involved in plant-growth promotion. Mol. Plant-Microbe Interact. 24:271-284.

Daims, H., Lebedeva, E. V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R. H., von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P. H., and Wagner, M. 2015. Complete nitrification by *Nitrospira* bacteria. Nature 528:504-509.

Day, D. A., Kaiser, B. N., Thomson, R., Udvardi, M. K., Moreau, S., and Puppo, A. 2001. Nutrient transport across symbiotic membranes from legume nodules. Funct. Plant Biol. 28:669-676.

Dixon, R., and Paiva, N. 1995. Stress-induced phenylpropanoid metabolism. Plant Cell 7:1085-1097.

Egamberdieva, D. 2009. Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. Acta Physiol. Plant. 31:861-864.

Erisman, J. W., Sutton, M. A., Galloway, J., Klimont, Z., and Winiwarter, W. 2008. How a century of ammonia synthesis changed the world. Nat. Geosci. 1:636-639.

Etesami, H., and Adl, S. M. 2020. Can interaction between silicon and non-rhizobial bacteria benefit in improving nodulation and nitrogen fixation in salinity-stressed legumes? A review. Rhizosphere 100229.

Filleur, S., Dorbe, M.-F., Cerezo, M., Orsel, M., Granier, F., Gojon, A., and Daniel-Vedele, F. 2001. An *Arabidopsis* T-DNA mutant affected in *Nrt2* genes is impaired in nitrate uptake. FEBS Lett. 489:220-224.

Fox, A. R., Soto, G., Valverde, C., Russo, D., Lagares, A., Zorreguieta, Á., Alleva, K., Pascuan, C., Frare, R., Mercado-Blanco, J., Dixon, R., and Ayub, N. D. 2016. Major cereal crops benefit from biological nitrogen fixation when inoculated with the nitrogen-fixing bacterium *Pseudomonas* protegens Pf-5×940: Robust biological nitrogen fixation in major cereal crops. Environ. Microbiol. 18:3522-3534.

Franche, C., Lindström, K., and Elmerich, C. 2009. Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35-59.

Fröhlich, A., Buddrus-Schiemann, K., Durner, J., Hartmann, A., and Von Rad, U. 2012. Response of barley to root colonization by *Pseudomonas* sp. DSMZ 13134 under laboratory, greenhouse, and field conditions. J. Plant Interact. 7:1-9.

Gautrat, P., Laffont, C., Frugier, F., and Ruffel, S. 2020. Nitrogen systemic signaling: From symbiotic nodulation to root acquisition. Trends Plant Sci. 26:392-406.

Giehl, R. F., Laginha, A. M., Duan, F., Rentsch, D., Yuan, L., and von Wirén, N. 2017. A critical role of AMT2;1 in root-to-shoot translocation of ammonium in *Arabidopsis*. Mol. Plant 10 1449-1460.

Girin, T., Lejay, L., Wirth, J., Widiez, T., Palenchar, P. M., Nazoa, P., Touraine, B., Gojon, A., and Lepetit, M. 2007. Identification of a 150-bp *cis*-acting element of the AtNRT2.1 promoter involved in the regulation of gene expression by the N and C status of the plant: Identification of *cis*-elements involved in the regulation of $NO_3^-$ uptake. Plant Cell Environ. 30:1366-1380.

Girke, C., Daumann, M., Niopek-Witz, S., Möhlmann, T. 2014. Nucleobase and nucleoside transport and integration into plant metabolism. Front. Plant Sci. 5.e00443.

Glick, B. R. 1995. The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. 41:109-117.

Glick, B. R. 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol. Res. 169:30-39.

Glick, B. R., and Bashan, Y. 1997. Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnol. Adv. 15:353-378.

Glick, B. R., Holguin, G., Patten, C. L., and Penrose, D. M. 1999. Biochemical and Genetic Mechanisms Used By Plant Growth Promoting Bacteria. World Scientific, London.

Hahlbrock, K., and Scheel, D. 1989. Physiology and molecular biology of phenylpropanoid metabolism. Ann. Rev. Plant Biol. 40:347-369.

He, S., Chen, M., Xie, Z., Yan, Y., Li, H., Fan, Y., Ping, S., Lin, M., and Elmerich, C. 2008. Involvement of GlnK, a PII protein, in control of nitrogen fixation and ammonia assimilation in *Pseudomonas stutzeri* A1501. Arch. Microbiol. 190:1-10.

Hirel, B., Tétu, T., Lea, P. J., and Dubois, F. 2011. Improving nitrogen use efficiency in crops for sustainable agriculture. Sustainability 3:1452-1485.

Howitt, S. M., and Udvardi, M. K. 2000. Structure, function and regulation of ammonium transporters in plants. BBA-Biomembranes 1465:152-170.

Iniguez, A. L., Dong, Y., and Triplett, E. W. 2004. Nitrogen fixation in wheat provided by *Klebsiella pneumoniae* 342. Mol. Plant-Microbe Interact. 17:1078-1085.

Jagtap, S., Trollman, H., Trollman, F., Garcia-Garcia, G., Parra-López, C., Duong, L., Martindale, W., Munekata , P. E. S., Lorenzo, J. M., Hdaifeh, A., Hassoun, A., Salonitis, K., and Afy-Shararah, M. 2022. The Russia-Ukraine conflict: Its implications for the global food supply chains. Foods 11:2098.

Jiménez, J. A., Novinscak, A., and Filion, M. 2020. Inoculation with the plant-growth-promoting rhizobacterium *Pseudomonas fluorescens* lbum677 impacts the rhizosphere microbiome of three oilseed crops. Front. Microbiol. 11:569366.

Kang, S.-M., Asaf, S., Khan, A. L., Lubna, Khan, A., Mun, B.-G., Khan, M. A., Gul, H., and Lee, I.-J. 2020. Complete genome sequence of *Pseudomonas psychrotolerans* CS51, a plant growth-promoting bacterium, under heavy metal stress conditions. Microorganisms 8:382.

Ke, X., Feng, S., Wang, J., Lu, W., Zhang, W., Chen, M., and Lin, M. 2019. Effect of inoculation with nitrogen-fixing bacterium *Pseudomonas stutzeri* A1501 on maize plant growth and the microbiome indigenous to the rhizosphere. Syst. Appl. Microbiol. 42:248-260.

Klessig, D. F., and Malamy, J. 1994. The salicylic acid signal in plants. Plant Mol. Biol. 26:1439-1458.

Klimczyk, M., Siczek, A., and Schimmelpfennig, L. 2021. Improving the efficiency of urea-based fertilization leading to reduction in ammonia emission. Sci. Total Environ. 771:145483.

Kloepper, J. W., Lifshitz, R., and Zablotowicz, R. M. 1989. Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol. 7:39-44.

Kwon, S. W., Kim, J. S., Park, I. C., Yoon, S. H., Park, D. H., Lim, C. K., and Go, S. J. 2003. *Pseudomonas koreensis* sp. nov., *Pseudomonas umsongensis* sp. nov. and *Pseudomonas jinjuensis* sp. nov., novel species from farm soils in Korea. Int. J. Syst. Evol. Microbiol. 53:21-27.

Lam, H.-M., Coschigano, K. T., Oliveira, I. C., Melo-Oliveira, R., and Coruzzi, G. M. 1996. The molecular-genetics of nitrogen assimilation into amino acids in higher plants. Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 47:569-593.

Larsen, P. E., Collart, F. R., and Dai, Y. 2015. Predicting ecological roles in the rhizosphere using metabolome and transportome modeling. PLoS One 10:e0132837.

Lejay, L., Tillard, P., Lepetit, M., Olive, F. D., Filleur, S., Daniel-Vedele, F., and Gojon, A. 1999. Molecular and functional regulation of two $NO_3^-$ uptake systems by N- and C-status of *Arabidopsis* plants. Plant J. 18: 509-519.

Li, W., Wang, Y., Okamoto, M., Crawford, N. M., Siddiqi, M. Y., and Glass, A. D. M. 2007. Dissection of the *AtNRT2.1*:*AtNRT2.2* inducible high-affinity nitrate transporter gene cluster. Plant Physiol. 143:425-433.

Liu, L. H., Ludewig, U., Frommer, W. B., and von Wirén, N. 2003. AtDUR3 encodes a new type of high-affinity urea/$H^+$ symporter in Arabidopsis. Plant Cell 15:790-800.

Liu, R., Zhang, Y., Chen, P., Lin, H., Ye, G., Wang, Z., Ge, C., Zhu, B., and Ren, D. 2017. Genomic and phenotypic analyses of *Pseudomonas psychrotolerans* PRS08-11306 reveal a turnerbactin biosynthesis gene cluster that contributes to nitrogen fixation. J. Biotechnol. 253: 10-13.

Mantelin, S., Desbrosses, G., Larcher, M., Tranbarger, T. J., Cleyet-Marel, J.-C., and Touraine, B. 2006. Nitrate-dependent control of root architecture and N nutrition are altered by a plant growth-promoting *Phyllobacterium* sp. Planta 223, 591-603.

Mérigout, P., Gaudon, V., Quilleré, I., Briand, X., and Daniel-Vedele, F. 2008. Urea use efficiency of hydroponically grown maize and wheat. J. Plant Nutr. 31:427-443.

Miflin, B. J., and Lea, P. J. 1980. Ammonia assimilation. Pages 169-202 in: Amino Acids and Derivatives. Academic Press, New York.

Mirza, M. S., Mehnaz, S., Normand, P., Prigent-Combaret, C., Moënne-Loccoz, Y., Bally, R., and Malik, K. A. 2006. Molecular characterization and PCR detection of a nitrogen-fixing *Pseudomonas* strain promoting rice growth. Biol. Fertil. Soils 43:163-170.

Mirza, M. S., Rasul, G., Mehnaz, S., Ladha, J. K., So, R. B., Ali, S., and Malik, K. A. 2000. Beneficial effects of inoculated nitrogen-fixing bacteria on rice. Pages 191-204 in: The Quest for Nitrogen Fixation in Rice. J. K. Ladha and P. M. Reddy, eds. International Rice Research Institute, Los Baños, Phillippines.

Muratore, C., Espen, L., and Prinsi, B. 2021. Nitrogen uptake in plants: The plasma membrane root transport systems from a physiological and proteomic perspective. Plants 10:681.

Mustafa, S. E. 2022. The importance of Ukraine and the Russian Federation for global agricultural markets and the risks associated with the current conflict. High Level Panel of Experts on Food Security and Nutrition, Food and Agriculture Organization of the United Nations, Rome.

Nadeem, S. M., Zahir, Z. A., Naveed, M., and Ashraf, M. 2010. Microbial ACC-deaminase: Prospects and applications for inducing salt tolerance in plants. Crit. Rev. Plant Sci. 29:360-393.

Okamoto, M., Kumar, A., Li, W., Wang, Y., Siddiqi, M. Y., Crawford, N. M., and Glass, A. D. M. 2006. High-affinity nitrate transport in roots of Arabidopsis depends on expression of the *NAR2*-like gene *AtNRT3.1*. Plant Physiol. 140:1036-1046.

Oliveira, A. D., Urquiaga, S., Döbereiner, J., and Baldani, J. I. 2002. The effect of inoculating endophytic $N_2$-fixing bacteria on micropropagated sugarcane plants. Plant Soil 242:205-215.

Orsel, M., Chopin, F., Leleu, O., Smith, S. J., Krapp, A., Daniel-Vedele, F., and Miller, A. J. 2006. Characterization of a two-component high-affinity nitrate uptake system in Arabidopsis. Physiology and protein-protein interaction. Plant Physiol. 142:1304-1317.

Owen, A. G., and Jones, D. L. 2001. Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. Soil Biol. Biochem. 33:651-657.

Padilla, F. M., Gallardo, M., and Manzano-Agugliaro, F. 2018. Global trends in nitrate leaching research in the 1960–2017 period. Sci. Total Environ. 643:400-413.

Panpatte, D. G., Shukla, Y. M., Shelat, H. N., Vyas, R. V., and Jhala, Y. K. 2017. Bacterial volatile organic compounds: A new insight for sustainable agriculture. Pages 151-166 in: Microorganisms for Green Revolution. Springer, Singapore.

Podile, A. R., and Kishore, G. K. 2007. Plant growth-promoting rhizobacteria. Pages 195-230 in: Plant-Associated Bacteria. S. S. Gnanamanickam, ed. Springer, Dordrecht, The Netherlands.

Poitout, A., Martinière, A., Kucharczyk, B., Queruel, N., Silva-Andia, J., Mashkoor, S., Gamet, L., Varoquaux, F., Paris, N., Sentenac, H., Touraine, B., and Desbrosses, G. 2017. Local signaling pathways regulate the Arabidopsis root developmental response to *Mesorhizobium loti* inoculation. J. Exp. Bot. 68:1199-1211.

Pratelli, R., and Pilot, G. 2014. Regulation of amino acid metabolic enzymes and transporters in plants. J. Exp. Bot. 65:5535-5556.

Prusiner, S., and Stadtman, E. R. 1973. The Enzymes of Glutamine Metabolism. Academic Press, New York.

Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V., and Samiyappan, R. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Prot. 20:1-11.

Ranathunge, K., El-Kereamy, A., Gidda, S., Bi, Y. M., and Rothstein, S. J. 2014. AMT1;1 transgenic rice plants with enhanced $NH_4^+$ permeability show superior growth and higher yield under optimal and suboptimal $NH_4^+$ conditions. J. Exp. Bot. 65:965-979.

Ravishankara, A. R., Daniel, J. S., and Portmann, R. W. 2009. Nitrous oxide ($N_2O$): The dominant ozone-depleting substance emitted in the 21st century. Science 326:123-125.

Raymond, J., Siefert, J. L., Staples, C. R., and Blankenship, R. E. 2004. The natural history of nitrogen fixation. Mol. Biol. Evol. 21:541-554.

Remans, T., Nacry, P., Pervent, M., Girin, T., Tillard, P., Lepetit, M., and Gojon, A. 2006. A central role for the nitrate transporter NRT2. 1 in the integrated morphological and physiological responses of the root system to nitrogen limitation in Arabidopsis. Plant Physiol. 140: 909-921.

Richardson, A. E. 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Funct. Plant Biol. 28: 897-906.

Rodionov, D. A., Dubchak, I. L., Arkin, A. P., Alm, E. J., and Gelfand, M. S. 2005. Dissimilatory metabolism of nitrogen oxides in bacteria: Comparative reconstruction of transcriptional networks. PLoS Comput. Biol. 1:e55.

Roychowdhury, R., Qaiser, T. F., Mukherjee, P., and Roy, M. 2019. Isolation and characterization of a *Pseudomonas aeruginosa* strain PGP for plant growth promotion. P. Natl. A. Sci. India B 89:353-360.

Rubio, L. M., and Ludden, P. W. 2005. Maturation of nitrogenase: A biochemical puzzle. J. Bacteriol. 187:405-414.

Rubio, L. M., and Ludden, P. W. 2008. Biosynthesis of the iron-molybdenum cofactor of nitrogenase. Annu. Rev. Microbiol. 62:93-111.

Ryu, C., Hu, C., Reddy, M. S., and Kloepper, J. W. 2003. Different signaling pathways of induced resistance by rhizobacteria in *Arabidopsis thaliana* against two pathovars of *Pseudomonas syringae*. New Phytol. 160: 413-420.

Saharan, B., and Nehra, V. 2011. Plant Growth Promoting Rhizobacteria: A Critical Review. Life Sci. Med. Res. 21:1-30.

Sajjad Mirza, M., Ahmad, W., Latif, F., Haurat, J., Bally, R., Normand, P., and Malik, K. A. 2001. Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane in vitro. Plant Soil 237:47-54.

Sanford, R. A., Wagner, D. D., Wu, Q., Chee-Sanford, J. C., Thomas, S. H., Cruz-García, C., Rodríguez, G., Massol-Deyá, A., Krishnani, K. K., Ritalahti, K. M., Nissen, S., Konstantinidis, K. T., and Löffler, F. E. 2012. Unexpected nondenitrifier nitrous oxide reductase gene diversity and abundance in soils. Proc. Natl. Acad. Sci. U.S.A. 109: 19709-19714.

Scotti, R., D'Agostino, N., and Zaccardelli, M. 2019. Gene expression profiling of tomato roots interacting with *Pseudomonas fluorescens* unravels the molecular reprogramming that occurs during the early phases of colonization. Symbiosis 78:177-192.

Setten, L., Soto, G., Mozzicafreddo, M., Fox, A. R., Lisi, C., Cuccioloni, M., Angeletti, M., Pagano, E., Díaz-Paleo, A., and Ayub, N. D. 2013.

Engineering *Pseudomonas protegens* Pf-5 for nitrogen fixation and its application to improve plant growth under nitrogen-deficient conditions. PLoS One 8:e63666.

Shinde, S., Cumming, J. R., Collart, F. R., Noirot, P. H., and Larsen, P. E. 2017. *Pseudomonas fluorescens* transportome is linked to strain-specific plant growth promotion in aspen seedlings under nutrient stress. Front. Plant Sci. 8:348.

Sieciechowicz, K. A., Joy, K. W., and Ireland, R. J. 1988. The metabolism of asparagine in plants. Phytochemistry 27:663-671.

Silby, M. W., Cerdeño-Tárraga, A. M., Vernikos, G. S., Giddens, S. R., Jackson, R. W., Preston, G. M., Zhang, X. X., Moon, C. D., Gehrig, S. M., Godfrey, S. A., Knight, C. G., Malone, J. G., Robinson, Z., Spiers, A. J., Harris, S., Challis, G. L., Yaxley, A. M., Harris, D., Seeger, K., Murphy, L., Rutter, S., Squares, R., Quail, M. A., Saunders, E., Mavromatis, K., Brettin, T. S., Bentley, S. D., Hothersall, J., Stephens, E., Thomas, C. M., Parkhill, J., Levy, S. B., Rainey, P. B., and Thomson, N. R. 2009. Genomic and genetic analyses of diversity and plant interactions of *Pseudomonas fluorescens*. Genome Biol. 10:1-16.

Smercina, D. N., Evans, S. E., Friesen, M. L., and Tiemann, L. K. 2019. To fix or not to fix: Controls on free-living nitrogen fixation in the rhizosphere. Appl. Environ. Microbiol. 85:e02546-18.

Smith, C., Hill, A. K., and Torrente-Murciano, L. 2020. Current and future role of Haber-Bosch ammonia in a carbon-free energy landscape. Energy Environ. Sci. 13:331-344.

Smith, C. J., Nedwell, D. B., Dong, L. F., and Osborn, A. M. 2007. Diversity and abundance of nitrate reductase genes (*narG* and *napA*), nitrite reductase genes (*nirS* and *nrfA*), and their transcripts in estuarine sediments. Appl. Environ. Microb. 73:3612-3622.

Song, B., and Tobias, C. R. 2011. Molecular and stable isotope methods to detect and measure anaerobic ammonium oxidation (anammox) in aquatic ecosystems. Meth. Enzymol. 496:63-89.

Sopanen, T., Burston, D., and Matthews, D. M. 1977. Uptake of small peptides by the scutellum of germinating barley. FEBS Lett. 79: 4-7.

Srivastava, S., Chaudhry, V., Mishra, A., Chauhan, P. S., Rehman, A., Yadav, A., Tuteja, N., and Nautiyal, C. S. 2012. Gene expression profiling through microarray analysis in *Arabidopsis thaliana* colonized by *Pseudomonas putida* MTCC5279, a plant growth promoting rhizobacterium. Plant Signal. Behav. 7:235-245.

Stajkovic, O., Delic, D., Josic, D., Kuzmanovic, D., Rasulic, N., and Knezevic-Vukcevic, J. 2011. Improvement of common bean growth by co-inoculation with *Rhizobium* and plant growth-promoting bacteria. Rom. Biotech. Lett. 16:5919-5926.

Suyal, D. C., Yadav, A., Shouche, Y., and Goel, R. 2014. Differential proteomics in response to low temperature diazotrophy of Himalayan psychrophilic nitrogen fixing *Pseudomonas migulae* S10724 strain. Curr. Microbiol. 68:543-550.

Torres, M. J., Simon, J., Rowley, G., Bedmar, E. J., Richardson, D. J., Gates, A. J., and Delgado, M. J. 2016. Nitrous oxide metabolism in nitrate-reducing bacteria. Adv. Microb. Physiol. 68: 353-432.

Trinh, C. S., Lee, H., Lee, W. J., Lee, S. J., Chung, N., Han, J., Kim, J., Hong, S.-W., and Lee, H. 2018. Evaluation of the plant growth-promoting activity of *Pseudomonas nitroreducens* in *Arabidopsis thaliana* and *Lactuca sativa*. Plant Cell Rep. 37:873-885.

Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T., and Singh, B. K. 2020. Plant-microbiome interactions: From community assembly to plant health. Nat. Rev. Microbiol. 18:607-621.

Urquhart, A. A., and Joy, K. W. 1981. Use of phloem exudate technique in the study of amino acid transport in pea plants. Plant Physiol. 68:750-754.

Venieraki, A., Dimou, M., Vezyri, E., Vamvakas, A., Katinaki, P.-A., Chatzipavlidis, I., Tampakaki, A., and Katinakis, P. 2014. The nitrogen-fixation island insertion site is conserved in diazotrophic *Pseudomonas stutzeri* and *Pseudomonas* sp. isolated from distal and close geographical regions. PLoS One 9:e105837.

Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571-586.

Walter, A., Römheld, V., Marschner, H., and Crowley, D. E. 1994. Iron nutrition of cucumber and maize: Effect of *Pseudomonas putida* YC 3 and its siderophore. Soil Biol. Biochem. 26:1023-1031.

Wang, H., Deng, N., Wu, D., and Hu, S. 2017. Quantitative response relationships between net nitrogen transformation rates and nitrogen functional genes during artificial vegetation restoration following agricultural abandonment. Sci. Rep. 7:7752.

Waterworth, W. M., and Bray, C. M. 2006. Enigma variations for peptides and their transporters in higher plants. Ann Bot. 98:1-8.

Wilton, R., Ahrendt, A. J., Shinde, S., Sholto-Douglas, D. J., Johnson, J. L., Brennan, M. B., and Kemner, K. M. 2018. A new suite of plasmid vectors for fluorescence-based imaging of root colonizing pseudomonads. Front. Plant Sci. 8:2242.

Yan, Y., Ping, S., Peng, J., Han, Y., Li, L., Yang, J., Dou, Y., Li, Y., Fan, H., Fan, Y., Li, D., Zhan, Y., Chen, M., Lu, W., Zhang, W., Cheng, Q., Jin, Q., and Lin, M. 2010. Global transcriptional analysis of nitrogen fixation and ammonium repression in root-associated *Pseudomonas stutzeri* A1501. BMC Genomics 11:1-13.

Yan, Y., Yang, J., Dou, Y., Chen, M., Ping, S., Peng, J., Lu, W., Zhang, W., Yao, Z., Li, H., Liu, W., He, S., Geng, L., Zhang, X., Yang, F., Yu, H., Zhan, Y., Li, D., Lin, Z., Wang, Y., Elmerich, C., Lin, M., and Jin, Q. 2008. Nitrogen fixation island and rhizosphere competence traits in the genome of root-associated *Pseudomonas stutzeri* A1501. Proc. Natl. Acad. Sci. U.S.A. 105:7564-7569.

Yang, Z., Han, Y., Ma, Y., Chen, Q., Zhan, Y., Lu, W., Cai, L., Hou, M., Chen, S., Yan, Y., and Lin, M. 2018. Global investigation of an engineered nitrogen-fixing *Escherichia coli* strain reveals regulatory coupling between host and heterologous nitrogen-fixation genes. Sci. Rep. 8:10928.

Yu, P., He, X., Baer, M., Beirinckx, S., Tian, T., Moya, Y. A. T., Zhang, X., Deichmann, M., Frey, F. P., Bresgen, V., Li, C., Razavi, B. S., Schaaf, G., von Wirén, N., Su, Z., Bucher, M., Tsuda, K., Goormachtig, S., Chen, X., and Hochholdinger, F. 2021. Plant flavones enrich rhizosphere Oxalobacteraceae to improve maize performance under nitrogen deprivation. Nat. Plants 7:481-499.

Zhang, T., Yan, Y., He, S., Ping, S., Alam, K. M., Han, Y., Liu, X., Lu, W., Zhang, W., Chen, M., Xiang, W., Wang, X., and Lin, M. 2012. Involvement of the ammonium transporter AmtB in nitrogenase regulation and ammonium excretion in *Pseudomonas stutzeri* A1501. Res. Microbiol. 163:332-339.

Zhuo, D., Okamoto, M., Vidmar, J. J., and Glass, A. D. 1999. Regulation of a putative high-affinity nitrate transporter (*Nrt2; 1At*) in roots of *Arabidopsis thaliana*. Plant J. 17 563-568.