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Differential contribution of plant-beneficial functions from *Pseudomonas kilonensis* F113 to root system architecture alterations in *Arabidopsis thaliana* and *Zea mays*

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Running title: Root architecture effects of F113 on plants mostly involve the plant beneficial functions encoded by *acdS* and *nirS* genes.

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SUMMARY

Fluorescent pseudomonads are playing key roles in plant-bacteria symbiotic interactions due to the multiple plant-beneficial functions (PBFs) they are harboring. The relative contributions of PBFs to plant-stimulatory effects of the well-known PGPR *Pseudomonas kilonensis* F113 (formerly *P. fluorescens* F113) were investigated using a genetic approach. To this end, several deletion mutants were constructed: simple mutants Δ*phlD* (impaired in the biosynthesis of 2,4-diacetylphloroglucinol [DAPG]), Δ*acdS* (deficient in 1-aminocyclopropane-1-carboxylate [ACC] deaminase activity), Δ*gcd* (glucose dehydrogenase deficient, impaired in phosphate solubilization), and Δ*nirS* (nitrite reductase deficient) and a quadruple mutant (deficient in the 4 PBFs mentioned above). Every PBF activity was quantified in the wild-type strain and the five deletion mutants. This approach revealed few functional interactions between PBFs *in vitro.* In particular, biosynthesis of glucose dehydrogenase severely reduced the production of DAPG. Contrariwise, the DAPG production impacted positively, but to a lesser extent, phosphate solubilization. Inoculation of the F113 wild-type strain on *Arabidopsis thaliana* Col-0 and maize seedlings modified the root architecture of both plants. Mutant strain inoculations revealed that the relative contribution of each PBF differed according to the measured plant traits, and that F113 plant-stimulatory effects did not correspond to the sum of each PBF relative contribution. Indeed, two PBF genes (Δ*acdS* and Δ*nirS*) had a significant impact on root system architecture from both model plants, whether in *in vitro* and *in vivo* conditions. The current work underlined that few F113 PBFs seem to interact between each other in the free-living bacterial cells, whereas they control in concert *Arabidopsis thaliana* and maize growth and development.

Key words: co-occurring plant-beneficial functions, maize, *Arabidopsis*, functional interactions, fluorescent *Pseudomonas*

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INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are root-colonizing bacteria able to provide growth and health benefits to plants through various mechanisms involving the expression of plant-beneficial functions (PBFs) in PGPR (Bruto et al. 2014; Lemanceau et al. 2017; Richardson et al. 2009; Vacheron et al. 2013). For example, PGPR can modulate plant hormonal signalization by producing phytohormones such as auxins, cytokinins, gibberellins, nitric oxide (NO) (Bashan and de-Bashan, 2010), or decreasing the emission of ethylene (Glick et al. 1998; Honma and Shimomura, 1978; Vacheron et al. 2016a). PGPR may also help plant nutrition by solubilizing inorganic compounds such as phosphate, or other mineral elements (Adesemoye and Kloepper, 2009; Vessey, 2003). Finally, some PGPR, in particular fluorescent pseudomonads, are producing antimicrobial compounds, such as 2,4-diacetylphloroglucinol (DAPG), that protects plants against plant pathogen microorganisms (Almario et al. 2014; Couillerot et al. 2009; Lanteigne et al. 2012) and activates plant defenses (Iavicoli et al. 2003). Some of these antimicrobial compounds can also act as communication signal between bacteria (Combes-Meynet et al. 2011).

The availability of an ever-increasing number of sequenced and annotated PGPR's genomes is allowing us to perform some comparative genomic analyses (Flury et al. 2016; Loper et al. 2012). The results of these analyses show the existence of combinations of PBF genes that are specific to some PGPR taxa (Bruto et al. 2014). For example, in strains or species belonging to the *Pseudomonas fluorescens* group, the genes required for the synthesis of DAPG are present simultaneously with the ones required for cyanid hydrogen (HCN) synthesis (Ramette et al. 2003). In *Pseudomonas protegens*, the production of pyoluteorin is co-occuring with the production of DAPG (Ramette et al. 2011). The presence of co-occurring PBF genes in genomes suggests that these functions might be part of common bacterial regulatory or metabolic networks. Alternatively, Bashan and de-Bashan (2010) hypothesized that each PBF present in one PGPR's genome would improve plant growth either simultaneously or successively. In this additive hypothesis, the overall improvement of plant growth would result from the sum of effects of each co-occurring PBF. Although the relative contribution of several antimicrobial compounds to biocontrol performance has been well studied (Calderón et al. 2013; Iavicoli et al. 2003; Romanowski et al. 2011; Voisard et al. 1989), the contribution of co-occurring PBFs in plant growth promotion is poorly documented.

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Among fluorescent pseudomonads, the *Pseudomonas kilonensis* F113 strain (formerly *Pseudomonas fluorescens;* Almario et al*.,* 2017) is a PGPR strain isolated from sugar beet rhizosphere (Shanahan et al. 1992). That strain is well known for its biocontrol activity against a broad range of plant pathogen microorganisms (Cronin et al. 1997a; Cronin et al. 1997b; Dunne et al. 1998). Less known, but all the more important, F113 also promotes plant growth and development, particularly on maize (Vacheron et al. 2016a; Walker et al. 2012), probably because of its rhizosphere's competence or in other words, its ability to efficiently colonize plant roots and to compete with other rhizosphere competent bacteria (Lugtenberg and Dekkers, 1999). The analysis of F113's genome sequence reveals the presence of multiple PBFs (Redondo-Nieto et al. 2013). We can identify the *phl* genes, which enable the production of DAPG (*phlD* encodes a polyketide synthase that is a key determinant of the DAPG biosynthesis), and also *gcd* encoding a glucose dehydrogenase involved in phosphate solubilization (Miller et al. 2010), *acdS* encoding the 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme (Prigent-Combaret et al. 2008; Vacheron et al. 2016a) and *nirS* encoding the nitrite reductase involved in NO production (Choi et al. 2006). Specific associations of co-occurring PBFs were frequently observed among *Proteobacteria* (Bruto et al. 2014), and may reflect the existence of functional interactions occurring between PBFs. In F113, the interaction between the 4 PBFs (i.e. DAPG production, ACC deamination, phosphate solubilization, and NO production) may happen at a regulatory or metabolic level and could result, when combined altogether, in enhanced plant growth. Following the additive hypothesis, we predict that the 4 co-occurring PBFs exert additive beneficial effects on the development of plant root system architecture (RSA) and on the overall growth of plants by *P. kilonensis* F113. To test this hypothesis, we used a genetic approach and constructed 5 mutant strains: 4 deleted for each PBFs, and on with deletion of the 4 PBFs. These 5 mutants were used to (i) analyze the functional relationships between F113 plant-beneficial activities and (ii) to determine if they are all equally contributing to the plant beneficial effects of F113 on *Arabidopsis thaliana* in *in vitro* conditions, and on maize (*Zea mays* L.) in soil pot experiment.

RESULTS

In vitro, most *P. kilonensis* F113's PBFs operate independently of each other

The existence of functional interactions between the 4 co-occurring PBFs of *P. kilonensis* F113 was first tested *in vitro* and in absence of plants. In the wild-type F113 strain (WT) and

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in each various mutant strains (i.e. Δ*acdS*, Δ*phlD*, Δ*nirS,* Δ*gcd* and the quadruple mutant strain), we measured the activity of ACC deamination, phosphate solubilization, nitrite reduction and DAPG synthesis. As expected, the deamination of ACC into α-ketobutyrate was impaired in the Δ*acdS* and quadruple mutants (**Fig. 1A**). AcdS activity was weakly induced in Δ*gcd* and Δ*nirS* compared with F113 (1.7-fold and 1.3-fold respectively) and not significantly affected in the Δ*phlD* mutant (**Fig. 1A**). Moreover, the insoluble phosphate was no longer solubilized in the Δ*gcd* and quadruple mutants (**Fig. 1B**). Furthermore, the glucose dehydrogenase Gcd activity was weakly but significantly decreased (1.7-fold) in the Δ*phlD* mutant but remained identical to the one of F113 in Δ*nirS* and Δ*acdS* (**Fig. 1B**). The Δ*nirS* and the quadruple mutants were unable to reduce nitrite (and consequently to produce NO) (**Fig. 1C**). The NirS activity in the Δ*acdS* or Δ*gcd* or Δ*phlD* strain was identical to the one of the F113 (**Fig. 1C**). As expected, there was no longer DAPG synthesis in the Δ*phlD* and quadruple mutants (**Fig. 1D**). Interestingly, there was a strong accumulation of DAPG in the Δ*gcd* (82 times more than F113). By contrast the Δ*acdS* and Δ*nirS* produced as much DAPG as F113 (**Fig. 1D**). Thus, the main functional interaction that occurs *in vitro* between all the four different PBFs is between the cellular pathways containing the glucose dehydrogenase Gcd and the polyketide synthase PhlD that both are involved in the control of DAPG biosynthesis in *P. kilonensis* F113.

To further study the interaction between DAPG synthesis and the glucose dehydrogenase, the DAPG production and phosphate solubilization were compared between all double and triple deletion mutants (**Fig. S1**). On one hand, no phosphate solubilization was observed in all *gcd* double and triple mutants. It is also significantly reduced in the Δ*phlD*Δ*acdS* (1.5-fold compared with F113), Δ*phlD*Δ*nirS* (1.3-fold compared with F113), and Δ*phlD*Δ*acdS*Δ*nirS* (1.7-fold compared with F113) mutants but in similar way than in the single Δ*phlD* mutant (1.7-fold compared with F113). Thus, apart from the small positive impact of DAPG production on the solubilization of phosphate, there is no other function that interacts with the latter (**Fig. S1**). On the other hand, DAPG production is impaired in all *phlD* double and triple mutants but is unaffected in a Δ*acdS*Δ*nirS* mutant (**Fig. S1**). Compared to the increase observed in a single *gcd* mutant (82 times higher than F113), DAPG production is much more increased in a Δ*acdS*Δ*gcd* mutant (226 times higher than F113) but reduced in the Δ*nirS*Δ*gcd* (30 times higher than F113) and Δ*acdS*Δ*gcd*Δ*nirS* mutants (26 times higher than F113)(**Fig. S1**). These results show that the cellular network involving the glucose

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dehydrogenase Gcd and the polyketide synthase PhlD is influenced negatively by *acdS* and positively by *nirS* (**Fig. S1**).

Deletion of PBF genes does not affect the fitness of *P. kilonensis* F113 *in vitro* but impair its rhizosphere soil competitiveness

When a PGPR does not enhance plant growth, it can be due to either from the absence of a particular PBF in that strain, the poor expression of the PBF gene in the rhizosphere or to a reduced rhizosphere competence. To assess if any of the tested PBF can affect rhizosphere competence, we measured the growth of the WT strain and of every single and quadruple mutant strains *in vitro* and *in vivo*. In liquid culture, bacterial growth can be limited by the time required to divide (characterized by the growth rate) and the size of the cells population in the stationary phase (characterized by the final OD reached in the liquid culture).

The growth rate of the quadruple mutant is higher than the one of the WT or any given single mutant strain (**Fig. 2, Table S1**). The growth rates of all simple mutants are similar to the one of the WT (**Fig. 2, Table S1**). In terms of final OD reached in stationary phase, the quadruple and the Δ*phlD* mutants reached the highest optical density in the stationary growth phase compared to any other single mutants and to the WT strain (**Fig. 2, Table S1**). Since neither the Δ*phlD* nor the quadruple mutant do synthesize DAPG *in vitro* (**Fig. 1D**), these results suggest that the production of DAPG might become toxic to F113 cells at the stationary phase, in the tested conditions. To test that hypothesis, the growth of F113, Δ*phlD* and its complemented mutant was evaluated in presence or absence of DAPG (**Fig. S2**). First, the complemented Δ*phlD* mutant had a growth kinetic curve similar to that of F113 (**Fig. S2A**). Second, the addition of DAPG reduced significantly the optical density in the stationary growth stage of F113 (starting from 0.1 $\mu g.ml^{-1}$ of DAPG) (**Fig. S2B**). Third, the growth of Δ*phlD* was slowed down in the exponential phase and reduced in the stationary growth by the addition of DAPG at 0.5 $\mu g.ml^{-1}$ and 1 $\mu g.ml^{-1}$ (**Fig. S2C**). Fourth, the addition of DAPG (at 0.5 $\mu g.ml^{-1}$ and 1 $\mu g.ml^{-1}$) slowed down the exponential growth stage of the complemented Δ*phlD* mutant, but the optical density reached in the stationary growth stage was quite similar to that of the complemented Δ*phlD* mutant and of F113 (**Fig. S2D**). Thus, when DAPG concentration reaches 0.5 $\mu g.ml^{-1}$, it negatively affects the growth of F113. Although the *gcd* mutant produced much more DAPG than the wild-type strain (and higher than 0.5 $\mu g.ml^{-1}$), its growth is nevertheless not affected. One can suggest that non-target effects of the *gcd* mutation might result in protection of the bacterium against high DAPG concentration. In any

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case, none of the simple and quadruple mutants have major deleterious effects on the F113 cell growth allowing us to analyze their role during the interaction of F113 with plant.

To monitor the contribution of PBF genes in the fitness of F113 in maize rhizosphere, F113 and mutants were inoculated on maize seeds grown in natural soil, in presence of the indigenous microbial communities. Same inoculation level of 5.10^6 bacteria per seed was used for each strain. F113 cell population was stable up to 8 dpi (days post-inoculation), but decreased at 20 dpi (**Fig. 3**). At 8 dpi, all mutant strains were found less abundant than F113 in the rhizosphere, the Δ*phlD* and quadruple mutants being the less numerous (**Fig. 3**). At 20 dpi, there was no significant difference in the rhizosphere colonization between F113 and the Δ*phlD* and Δ*gcd* mutants, whereas Δ*acdS*, Δ*nirS* and the quadruple mutants were less abundant than F113 (**Fig. 3**). Thus, all PBFs contribute to F113 rhizosphere competence but to various extends. In particular, *phlD* is relevant for root colonization up to 8 days and *acdS* and *nirS* genes are important for ensuring effective maize colonization over 20 days.

The *acdS*, *nirS* and *gcd* genes affect maize growth

F113 and PBF mutants were inoculated on maize seeds grown in pot filled with soil. Twenty days after, shoot and root architecture traits were measured and the profiles obtained for each strain were compared using hierarchical clustering analyses (HCA). Compared to the non-inoculated condition, F113 significantly enhanced root growth (with an increase of total root length of +50%, and of total root surface of +46%) and shoot growth (with an enhancement of the total leaf surface of +44%) (**Tables S2, S3** and **Fig. S3**).

When comparing the inoculation effects of mutants to that of F113 on maize by HCA, two clusters were formed, grouping F113, Δ*phlD* and Δ*gcd* on one hand and Δ*acdS,* Δ*nirS* and the quadruple mutant in the other hand (**Fig. 4**). In the first cluster, all the plant parameters appear evenly affected during inoculation, giving rise to a shape like a circle similar to the one obtained with F113. It suggests that there are either no difference or minor differences between F113 and the Δ*phlD* and Δ*gcd* mutants. Consistent with that, there is no significant differences between F113 and Δ*phlD* for all plant parameters assessed (**Table S2** and **S3**), while the inoculation with Δ*gcd* reduced significantly maize root length and surface with respectively 26% and 20% of decrease compared to F113 (**Table S2**).

In the second cluster, the plant parameters appear to be unevenly affected, giving rise to an unsymmetrical shape. The three strains belonging to that cluster (i.e. Δ*acdS,* Δ*nirS* and the quadruple mutant have a similar impact on maize development and growth (**Table S2** and

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S3). Contrasting with the strains of the first cluster, the inoculation of Δ*acdS,* Δ*nirS* and the quadruple mutant impacted significantly the root system architecture compared to F113 by reducing significantly the root length (-64%, -59% and -71%, respectively), the root surface (-50%, -50% and -57%, respectively), the number of root tips (respectively -62%; -45% and -71%) and the root fresh weight (respectively -31%, -38% and -42%) (**Table S2**). Thus, the RSA modifications triggered by F113 on maize were lost when Δ*acdS,* Δ*nirS* or the quadruple mutants were inoculated (**Tables S2, S3** and **Fig. S3**).

In conclusion, F113's PBF genes and in particular *acdS* and *nirS* appear to alter mostly maize root parameters but not shoot parameters.

F113 PBFs differently affect *A. thaliana* root hair elongation and primary root growth in gnotobiotic agar conditions

To further investigate what is F113's PBF impact on root, we turned to *in vitro* experiments, as they allow a careful monitoring of F113's effects on root development or root hairs for example. Because maize is a large plant, with a root system that is likely to be too large to be evaluated *in vitro*, we turned instead to *Arabidopsis*, as its root development shares some similarities with the one of maize (Atkinson *et al*., 2014; Hochholdinger and Zimmermann, 2008).

We first verified the effect of F113 inoculation and that of the various mutant strains on the *Arabidopsis* primary root growth. F113 inoculation inhibited significantly (2-fold) the primary root length of *A. thaliana* (**Fig. 5A**). When *A. thaliana* Col-0 was inoculated with Δ*phlD,* a smaller reduction of the primary root growth (1.5-fold) compared to F113 was observed. However, the difference with F113 was not significant, implying that DAPG production only weakly contributed to the primary root length inhibition triggered by the WT strain (**Fig. 5A**). Contrarily, primary root length inhibition was lost when Δ*acdS*, Δ*gcd*, Δ*nirS* or the quadruple mutants were inoculated (**Fig. 5A**). Therefore, *acdS*, *gcd* and *nirS* independently contribute to *Arabidopsis* primary root growth inhibition driven by F113 (**Fig. 5A**). Secondly, we investigated the effect of F113 and of the various mutant strains on the plant root hair length. F113 inoculation led to significantly longer root hairs (**Fig. 5B**). Inoculation with the quadruple mutant generated the same plant phenotype than the non-inoculated condition, suggesting that at least one of the deleted PBFs was responsible of enhanced root hair length (**Fig. 5B**). However, compared to F113 WT inoculated plants, the average root hair lengths were significantly reduced by 20% in Δ*phlD*, 14% in Δ*acdS*, 21% in Δ*gcd* inoculated plants, and up 41% in Δ*nirS* inoculated plants (**Fig. 5B**). Therefore, none of

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F113's PBF functions is solely controlling root hair elongation. Because the activation of the plant ethylene signaling pathway results in root growth inhibition and root hair elongation (Pitts *et al*., 1998; Swarup et al. 2007), and is also an indicator of the activation of a defense related mechanism (Broekgaarden *et al*., 2015), we looked at the effects of F113 and of the various mutants strains on the plant ethylene emission by photoacoustic infra-red spectrophotometry (Cristescu *et al*., 2012). Plant ethylene emissions increased significantly when F113 (1.5 fold), and Δ*acdS* (2 fold) were inoculated comparatively to the non-inoculated plant. The plant ethylene emission produced by Δ*phlD,* Δ*gcD*, Δ*nirS* and the quadruple mutant did not significantly differ from that of the non-inoculated condition (**Fig. 6**). In conclusion, all F113's PBFs are contributing to the production of ethylene by *Arabidopsis,* but in a distinct manner: *acdS* is reducing *Arabidospis* ethylene content whereas *phlD*, *gcd* and *nirS* are increasing it.

DISCUSSION

In this work, we evidence that interactions between PBFs might be different when bacteria are growing *in vitro* or in contact with a plant, where functional overlapping between PBFs may occur by affecting the same plant pathway. It highlights that inactivation of one PBF gene can affect the activity of other ones and that all PBFs harboured by PGPR have to be taken into account when analysing the plant response effects of PGPR inoculation.

Functional interactions between co-occurring PBFs in *P. kilonensis* F113

In vitro, the main functional interaction occurring in F113 cells between the four studied PBFs is between phosphate solubilization and biosynthesis of DAPG. We evidenced that the glucose dehydrogenase activity (Gcd) is repressing DAPG biosynthesis in F113 (**Fig. 1D**), confirming observations formerly made in *P. protegens* CHA0 (de Werra et al*.,* 2009). In cells containing a functional Gcd, glucose-6P is transformed into gluconic acid, which is exported out of the cells, leading to medium acidification and phosphate solubilization (**Fig. S4A**) (Stella and Halimi, 2015). When *gcd* is deleted, a higher quantity of glucose-6P could be provided to the glycolysis, resulting on the one hand in increased ATP production by the cell, and on the second hand in a higher amount of pyruvate that could then be incorporated to the tri-carboxylic acid cycle to produce higher amount of acetyl CoA, the precursor of DAPG (Bangera and Thomashow, 1999; Clifford et al. 2015) (**Fig. S4B**). Interestingly, a metabolic

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interaction involving Gcd with another antimicrobial compound (i.e. pyoluteorin) has been evidenced in *P. protegens* CHA0 (de Werra et al. 2009).

DAPG was shown to act as an intracellular messenger controlling transcription of different PBFs genes such as pyoluteorin production in *P. protegens* CHA0 and Pf-5 (Clifford et al. 2015; de Werra et al. 2011; Kidarsa et al. 2011; Schnider-Keel et al. 2000) or as an intercellular messenger inducing PBFS in other PGPR as auxin production (Combes-Meynet et al. 2011). Therefore, we hypothesize that the Δ*gcd* mutant strain could also show some specific modification at the transcriptional level of the expression of other PBFs encoding genes, resulting in potential functional compensation.

Distinct roles for F113 PBFs during colonization

The functional importance of F113's PBF functions and its competitiveness against the native soil populations of rhizobacteria was tested on maize plants growing in pot filled with non-sterile soil, a setup close to natural conditions. For this experiment, we selected maize as a model because it is frequently grown in fields where the soil we used has been collected suggesting that this soil is enriched in a maize specific rhizosphere microbiota that is ideal to compare the rhizosphere competitiveness of a mutant *vs* a wild-type strain. In addition, maize plantlets have a large root system allowing an easy quantification of the colonization process.

Root secretion of organic substances is essential for the rhizosphere microbiota to thrive (Haichar et al. 2016). Since the root exudates are composed of a lot of organic molecules, which origin can be traced back to the photosynthetic activity of the plant, it is important that the two time points selected to sample the maize root rhizosphere will not differ in the physiological state of the plant. We sampled maize roots 8 days and 20 days after sowing. In both case, the maize seedlings are already performing photosynthesis and may release root exudate whose composition is not drastically dissimilar. Consistent with this hypothesis, the composition in secondary metabolites present in root exudates from 5 distinct maize inbred lines is less different between 8 and 10 days than it is between 3 and 5 days post-germination (Walker and Prigent-Combaret, unpublished results).

At 8 dpi, there are less Δ*phlD* mutant bacteria in the maize root rhizosphere than F113 while at 20 dpi, the Δ*phlD* mutant strain is equally abundant as the WT (**Fig. 3**). This suggests that DAPG is important at the beginning of the colonization process. DAPG is a well-known antimicrobial compounds secreted by F113 (Cronin et al. 1997). Its secretion could limit the

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presence of competitor rhizobacteria or fungi *in vivo*, hence favoring an exclusive interaction between F113 and the host roots. Curiously, the Δ*gcd* mutant strain is equally abundant as the WT strain at both time points (**Fig. 3**). Yet, the Δ*gcd* mutant strain produces 3 times more DAPG *in vitro* than the WT strain (**Fig. 1**). Enhanced DAPG production may trigger some compensation mechanisms in the Δ*gcd* mutant strain, allowing it to colonize maize roots as efficiently as the wild-type strain.

At 20 dpi the colonization of the Δ*nirS,* Δ*acdS* and the quadruple mutant is less efficient than that of F113, Δ*gcd*, and Δ*phlD* (**Fig. 3**). $AcdS^+$ strains are catabolizing ACC exuded by plant roots in α-ketobutyric acid (carbon source) and NH_4^+ (nitrogen source) (Glick et al. 1998). *acdS* deletion can reduce the ability of F113 to fulfill its nutrient requirements in the rhizosphere, and thus its fitness and root colonization ability. NirS is allowing bacteria of the *Pseudomonas* genus, a strictly respiring genus, to respire NO_3^- and NO_2^- in the microaerophilic or anaerobic conditions that are frequently encountered in the rhizosphere (Højberg et al. 1999). Thus, the loss of nitrogen respiring capacity may explain the lower root colonization ability of the Δ*nirS* mutant that has previously been observed (Ghiglione et al. 2002) as well as in our condition (**Fig. 3**). Since both NirS and AcdS are important for the colonization at 20 dpi, one could have expected that in the quadruple mutant strain, the effects of the two mutations would add up resulting in a strain with very poor ability to colonize at 20 dpi. Surprisingly, this is not the case (**Fig. 3**). This suggests the existence of potential epistatic interactions between the two genes *nirS* and *acdS* or with *gcd* and/or *phlD* that our *in vitro* analysis failed to unravel (**Fig. 1**). It also might be that plant exudates or microbe-microbe interactions in the rhizosphere result in activation of other genes that compensate for the lack of *nirS* and *acdS*.

The maize colonization process by F113 has a significant impact on the plant root and shoot growth.

To test if these different colonization patterns could affect maize growth *in vivo*, we measured 15 different physiological parameters (8 for shoots and 7 for roots; **Table S2 and S3**). To get a broad view of the effect of F113 on maize plants, we have used a hierarchical clustering analysis (HCA). Instead of focusing on each parameter, HCA takes into account parameter patterns that can be compared between the mutant and the wild-type strains (**Fig. 4**). The inoculation of maize with the Δ*phlD* or Δ*gcd* strains resulted in a pattern similar to the one of F113 (**Fig. 4**). Since *phlD* appears to play a role for maize root colonization at 8 dpi (**Fig. 3**), it suggests that F113 colonization of maize roots after 8 dpi is more important for the effects

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on maize growth than up to 8 dpi. Consistent with that interpretation, the inoculation of maize roots with the Δ*nirS* or Δ*acdS* or the quadruple mutant strain (all these genes are important for the colonization at 20 dpi (**Fig. 3**)), is resulting in a pattern different from the one of the WT (**Fig. 4**).

Contrasting with F113, inoculation with Δ*acdS* or Δ*nirS* or the quadruple mutant resulted in a decrease in total root length, total root surface and number of root tips while at the same time, an increase in root diameter (**Fig. 4, Table S2**). This indicates that *acdS* and *nirS,* two genes that are important for the fitness of F113, are also important for the root growth promotion. How they could be beneficial to root maize growth remains unclear. For example, it could be a direct interference with the plant ethylene pathway for AcdS or with the plant NO pathways for NirS. At the level of the shoot, maize plants inoculated with Δ*acdS* or Δ*nirS* or the quadruple mutant show a weak decrease in leaf surfaces, as well as in chlorophyll content compared to F113 (**Fig. 4; Table S3**). While the leaf surface of inoculated maize plants by Δ*acdS*, Δ*nirS* or the quadruple mutant are slightly higher than in the non-inoculated plants, the chlorophyll content is lower. As chlorophyll fluorescence is reduced in stressed plants (Bolhàr-Nordenkampf and Öquist, 1993), this result could suggest that maize plants inoculated with Δ*acdS* or Δ*nirS* or the quadruple mutant are stressed, whereas F113 may alleviate plant stress. Taken together, the PBF functions that contribute to the long-term fitness of F113 are also the ones that have the strongest effects on the growth of maize plants.

Relative contribution of PBFs on *A. thaliana* root architecture

To further study the contribution of the F113 various PBF's on the root development, we performed *in vitro* experiments with plants. We have used *Arabidopsis* as a model plant because it is small, it grows fast, it has a relatively simple root development program (tap root), and its root hairs are very easily observable. In addition, maize and *Arabidopsis* root development programs share some similarities (Atkinson *et al*., 2014; Hochholdinger and Zimmermann, 2008). Finally, F113, which has been initially isolated from sugar beet roots is known to colonize efficiently both monocotyledons such as wheat (Couillerot et al. 2011; De La Fuente et al. 2006) and maize over more than 5 weeks (Couillerot et al. 2013; Von Felten et al. 2010) but also dicotyledonous crops such as alfalfa (Sanchez-Contreras et al. 2002; Villacieros et al. 2003) or tomato (de Weert et al. 2002).

In vitro F113 inoculation resulted in *Arabidopsis* primary root growth inhibition and strong promotion of root hair elongation (**Fig. 5A and B**). By contrast, the quadruple mutant showed no root architecture difference compared to the non-inoculated *Arabidopsis*,

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suggesting that at least one F113 PBFs or a combination of PBFs may trigger root architecture modifications (**Fig. 5, Fig. S3**). Several plant hormones such as auxin (controlling cell division and elongation) (Spaepen and Vanderleyden, 2011) or ethylene (controlling root cell elongation) (Jung and McCouch, 2013; Street et al. 2015; Swarup et al. 2007;) control primary root elongation. However, F113 does not produce auxinic compounds (Vacheron, Muller and Prigent-Combaret unpublished results) but instead is producing DAPG (**Fig. 1**). One possibility is that DAPG could have an effect on the plant root system. Consistent with this, when tomato seedlings are growing *in vitro* and in presence of 10µM DAPG, their primary root growth is inhibited (Brazelton et al. 2008). However, the Δ*phlD* mutant (none producing DAPG) remains able to inhibit *Arabidopsis* primary root elongation and the Δ*gcd* mutant (over-producing DAPG) does not show further reduced primary root length compared to F113 (**Fig. 5**). In addition, both the Δ*phlD* and Δ*gcd* mutants remain able to stimulate root hair length. These results imply that, aside from DAPG, other F113 PBFs are involved in the inhibition of the primary root elongation and stimulation of root hair length in *A. thaliana.* Interestingly, the loss-of-function mutation in *nirS* affects the inhibition of primary root growth (**Fig. 5A**), and the root hair elongation (**Fig. 5B**) significantly. This could suggest that NO, produced by F113's NirS activity as evidenced by the use of the specific fluorescent probe diaminorhodamine-4M acetoxymethyl ester (Vacheron, Muller and Prigent-Combaret unpublished results), would act as an inhibitor of primary root growth and activator of root hair elongation. Consistent with this, NO is described as a positive regulator of root hair development (Lombardo et al. 2006; Lombardo and Lamattina, 2012) and a negative regulator of primary root growth (Fernández-Marcos et al. 2011).

In absence of PGPR, plant ethylene is known to inhibit primary root length and to activate root hairs elongation (Cao et al. 1999; Pitts et al. 1998). F113 moderately increased plant ethylene emission, whereas all mutants but Δ*acdS,* shared similar plant-ethylene emission than non-inoculated condition (**Fig. 6**). Individually, each of the F113 PBFs contributes positively to ethylene production by the plant during the associative symbiosis with *A. thaliana* (**Fig. 6**). Contrasting with the other mutants, the Δ*acdS* strain colonization results in higher ethylene emission by the plant (**Fig. 6**). Hence, the AcdS activity negatively contributes to ethylene production. In our study, this mutant strain is the only one simple mutant unable to degrade *in vitro* the ethylene precursor ACC (**Fig. 1A**). Assuming that the mutant strain behaves similarly *in vitro* and *in vivo*, the lack of AcdS activity result in higher ethylene emission by the plant (**Fig. 6)**. However, this increase is not observed with the

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inoculation of the quadruple mutant, showing that the other PBFs activate plant ethylene production. Thus, it confirms that F113 colonization of the root system results in modulation of ethylene emission by the plant and that AcdS is not the only one F113's PBF contributing to this effect. However, it remains to be seen if this emission is greater in the root, in the shoot or both plant organs. It also confirms that AcdS activity is actively decreasing plant ethylene emission during the associative symbiosis. This result is consistent with a long-standing hypothesis on the function of AcdS during its interaction with plants (Glick et al. 1998). Yet, this increased emission of ethylene in presence of the Δ*acdS* mutant strain does not result in a shorter primary and longer root hairs compared to the effect of the WT strain (**Fig. 5A**, and **B**). This absence of stronger root responses contrasts with previous observations suggesting that an absence of AcdS activity resulted in longer root hairs (Galland et al. 2012). But *Phyllobacterium brassicacearum* STM196, *Rhizobium leguminosarum* 128C53K and *Pseudomonas* sp. UW4, the strains used in that previous work, may not have the same combination of PBFs as the one of F113. Therefore, one should not rule out the possibility that this absence of stronger root responses with the Δ*acdS* strain is specific to F113 and its combination of PBFs. Another possibility that could explain the absence of stronger root responses, despite a significant increase in ethylene emission, is that F113 Δ*acdS* inoculated plants are becoming insensitive to the effect of high ethylene on root cells.

Taken together, F113 may trigger both ethylene-dependent and NO-dependent signalling pathways on *Arabidospsis*. Contrasting with the observations made on soil-pot grown maize, all F113's PBFs tested *in vitro* on *Arabidopsis* contribute to modification of the root architecture.

EXPERIMENTAL PROCEDURES

Bacterial strains, plasmids and culture conditions

The bacterial strains and plasmids used in this study are listed in (**Table S4)**. *P. kilonensis* F113 wild-type and its mutants were incubated at 28°C in King's B broth media (Simon and Ridge, 1974) supplemented when required with gentamycin (15 $\mu g.ml^{-1}$) or kanamycin (Km; 50 $\mu g.ml^{-1}$). *Escherichia coli* was incubated at 37°C in Luria Bertani broth (Bertani, 1951) supplemented with gentamycin (15 $\mu g.ml^{-1}$) or ampicillin (75 $\mu g.ml^{-1}$) and kanamycin (Km; 50 $\mu g.ml^{-1}$) when necessary. In order to assess the effects of DAPG on the growth of F113

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and its mutants, DAPG (Santa Cruz Biotechnology, USA) was added in King's B broth medium at 3 different concentrations (0.1 $mg.ml^{-1}$, 0.5 $mg.ml^{-1}$, and 1 $mg.ml^{-1}$).

Growth kinetic assays

Bacterial growth was monitored during 24 h at 28°C by measuring optical density (OD) in a Bioscreen C analyzer (Labsystems, Finland) with one OD 600nm measured every 15 min. Growth kinetic assays were performed three times independently with 5 technical repetitions.

Construction of deletion mutants and complementation of the *phlD* deletion mutant

To construct F113 PBF deletion mutants, about 700-pb fragments were amplified from regions upstream and downstream the PBF genes of interest using specific primers (**Table S5**) and subcloned into pGEM®-T easy (Promega, Madison, USA). The upstream and downstream DNA fragments were digested with AatII/MunI and SacII/SacI for Δ*phlD* and Δ*gcd*, AatII/PvuII and SacII/SacI for Δ*acdS*, PvuII/MunI and SacII/SacI for Δ*nirS* and then cloned one after another into pCM184, on both sides of the kanamycin resistance encoding gene (Marx and Lidstrom, 2002). All restriction enzymes were supplied by ThermoFisher Scientific (USA). All plasmid amplifications were done using *E. coli* JM109 chemically competent cells (Promega, Madison, USA). For validation of the correct insertion of upstream and downstream DNA fragments into pCM184, sequencing using primers S1F/S1R and S2F/S2R was performed. pCM184 harboring upstream and downstream regions of the plant-beneficial genes of interest were transformed into F113 by electroporation (Højberg et al. 1999). Inactivation mutants resulting from a double recombination event of upstream and downstream regions were selected on King's B Km and screened for plant-beneficial gene deletion by PCR with specific primers. Then, the plasmid CM157 containing the *cre-lox* recombinase gene was introduced into F113 kanamycin resistant mutants in order to remove the antibiotic marker. To check the loss of the kanamycin cassette, mutant strains were screened for their inability to grow on King's B Km and by PCR using external primers. For complementation of the *phlD* deletion mutant, the coding region of the *phlD* gene of F113 was amplified by PCR using primers phlDCompF1 and phlDCompR1 and subcloned into pGEM-T easy. The *phlD* gene was extracted from pGEM-T easy using ApaI/SacI digestion and inserted into the multiple cloning site of vector pBBR1MCS-5, under control of the lac

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promoter, resulting in pBBR1-MCS5:*phlD*. This plasmid was introduced into the Δ*phlD* mutant by electroporation (Højberg et al. 1999).

DAPG quantification by HPLC

DAPG production was measured by HPLC according to the method developed by (Bonsall et al. 1997). Briefly, samples were analyzed with an Agilent 1200 series HPLC (Agilent Technologies, Santa Carla, USA) equipped with a degasser (G132A), a quaternary pump module (G1311A), an automatic sampler (G1329A) and a diode array detector (DAD G1315B). Separation of compounds was performed at room temperature with a C18 reverse-phase column. For each sample, 20 µl were injected and eluted at 1 $ml.min^{-1}$ using a step-by-step gradient increasing acetonitrile (ACN) proportion in water: the gradient started at 40% of ACN over 4 min then rose from 40 to 64% in 7.5 min, then came to 75% at 16.5 min and ended at 100% at 18.5min (100% maintained for 3 min before decreasing to 40% for 5 min). Chromatograms were recorded at 270nm (maximum of absorbance of DAPG). The chemstation Agilent software was used for integration of chromatograms, and quantitation of DAPG was done according to a reference curve with the chemical standard (Sigma-Aldrich). This experiment was done independently three times.

ACC deaminase activity assay

The ACC deaminase activity of strains was performed as described by Blaha et al. (2006). The method is based on the quantification of α-ketobutyrate produced by the deamination of ACC into α-ketobutyrate by the enzyme ACC deaminase. Total protein content in the assay was measured using the BCA Protein Assay Kit (Pierce, Rockford, IL). ACC deaminase activity was assessed three times for each strain. The data are expressed in nmol of ketobutyrate.h^{-1}.mg of $protein^{-1}$. This experiment was done independently three times.

Phosphate-solubilizing assay

The phosphate-solubilizing ability of strains was evaluated by measuring tricalcium phosphate solubilization halos on a National Botanical Research Institute's Phosphate (NBRIP) agar medium. Strains were cultured for 1 day at 28°C in King's B broth and then deposited 4 times (5 µl each) on NBRIP agar medium. Solubilization halos were measured

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after 6 days of incubation at 28°C using ImageJ software. This experiment was done three times independently.

Denitrification activity measurement

The denitrification activity was estimated through the potential accumulation of N_2O in the culture media, which is one step of the denitrification process. The accumulation of N_2O was measured according to Bardon et al. (2016). Briefly, 20 ml of King's B media supplemented with 20 mM of KNO_3 were placed in a plasma flask sealed by a rubber stopper. Anaerobic condition was obtained removing air and replaced it with He/C_2H_2 mixture (90/10; v/v) in order to inhibit the reduction of N_2O to N_2 and to allow the N_2O accumulation. Each strain was inoculated at a final OD_{600} of 0.1. N_2O emissions were measured every 2h during 24h using gas chromatography coupled to a micro-katharometer detector (µGC-R3000, SRA instruments, Marcy l'Etoile, France). The assay was performed twice with two technical replicates. N_2O emission was expressed as µg of $N\text{-}N_2O.h^{-1}.ml^{-1}$ of media. In addition, the ability of F113 to produce NO intracellularly was evaluated using the specific fluorescent probe diaminorhodamine-4M acetoxymethyl ester (Calbiochem), as previously described by Pothier et al. (2007).

Bacterial inoculum preparation

The *P. kilonensis* F113 wild-type and related deletion mutants Δ*phlD,* Δ*acdS*, Δ*gcd*, Δ*nirS* and the quadruple mutant (Δ*phlD*Δ*acdS*Δ*gcd*Δ*nirS*) were incubated overnight at 28°C in King's B broth medium. Cell cultures were centrifuged at 3000 rpm. The pellet was washed with sterilized water and adjusted to 10^7 bacteria.ml^{-1}.

Bacterial inoculation effects on *Arabidopsis* root system architecture

The seeds of *Arabidopsis thaliana* Ecotype Columbia0 (Col-0) were prepared according to (Mantelin et al. 2006). After 7 days, plantlets were transferred to 50 ml of new plant agar medium containing F113 or mutant strains at 10^7 cells.ml^{-1} in square Petri dishes (120 x 120 mm). Inoculated plantlets were transferred to a phytotron set to the following parameters: 21°C/days; 17°C/night, 13h light periods, 9500 lux. Four *Arabidopsis* plantlets were disposed per Petri dish and 6 plates were prepared per condition. The 7 conditions tested were *A. thaliana* inoculated with F113, with the 4 simple mutants, with the quadruple mutant and the

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non-bacterized control condition. This experiment was made thrice independently. After 7 days, 3 plants per Petri dishes were scanned in order to analyze the primary root elongation and root hair length using ImageJ software (i.e. 18 analyzed plants per condition). The relative contribution of each tested PBF in the modulation of the *Arabidopsis* root architecture was calculated as follow:

(1) F113 – control = α

(2) ((F113 – mutant) / α) x 100 = deleted gene relative contribution to F113 effect (%)

Arabidopsis ethylene emission

Measurements of *in vivo* ethylene emission by *Arabidopsis* plantlets were performed using infrared laser spectroscopy coupled to photo-acoustic detection (Cristescu et al. 2012) (ETD-300, SensorSense, Nijmegen, Holland). Practically, we carefully poured 600 µl of sterile plant culture medium (as described above) inoculated or not with the various F113 strains at a final density of $2x10^7$ cells.ml^{-1} in a 2 ml sterile Eppendorf tube. Once solidified, we carefully introduced two wild-type *Arabidopsis* plantlets aged of seven days and closed the lid. Lids were punched to allow a regular breathing of the plants. This system was then transferred to a phytotron (MLR-352-PE, Panasonic, Gunma, Japan) set to the following parameters: 21°C (day); 17°C (night), 13h light period, 9500 lux. No measure was made during the first 48 h to allow plant acclimation, and in order to recover stress associated to the transfer. Prior ethylene emission measurement, 200 µl of plant liquid medium was added in each tube in order to keep the head space humid, and therefore protecting the plant from the dry air coming from the compressed air bottle. Then, the lids of the tubes were removed and replaced by a rubber plug. Needles were pushed through the plug to allow the headspace to be flushed to the ethylene detector by compressed air. Every 90 min, the ethylene content in the headspace was measured while the tubes remained in the phytotron. That measurement was performed during two to three consecutive days to collect enough data on ethylene content during the day. At the end of the experiment, plant fresh weight was measured to allow data normalization. Raw results were exported into a spreadsheet in which they were normalized and processed to obtain at least 10 different measures of ethylene content in presence of light in each condition.

Maize inoculation experiments in non-sterile soil

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The semi-late maize hybrid PR37Y15 (Pioneer Semences, Aussonne, France) was used in this study. Maize seeds were surfaced disinfected. Briefly, seeds were soaked in sodium hypochlorite (3.4%) during one hour with agitation. Five washes were performed with sterile water. Four maize seeds were sown in non-sterile soil taken from La Côte Saint-André (LCSA) near Lyon (France; Vacheron et al. 2016a) and placed in 2dm^3 pots filled with 2.0kg of sieved soil (5mm) with a water content of 20% (v/v). The moisture was maintained at 20% during the experiment. Seeds (4 per pot) were inoculated with 100 µl of bacterial inoculum solution, corresponding to 10^6 bacteria. Ten pots per conditions were performed. Inoculum impact on plant growth was assessed at 20 dpi by characterizing maize root system architecture using WinRhizo (Reagent Instruments, Quebec City, Quebec, Canada). Leaf surface was measured with the WinFolia image analysis software (Regent Instruments, Quebec City, Quebec, Canada). Chlorophyll content was measured with a chlorophyll content meter (Hansatech instruments model CL-01, Amesbury, USA). Root and shoot fresh and dry biomasses were weighed. Ten plants were analyzed per conditions.

Monitoring the bacterial maize root colonization

The measurement of root colonization was assessed at 0, 8 and 20 dpi taking 10 seeds for each condition at each sampling time. Inoculated seeds or plants were harvested and disposed in a 50 ml Falcon tube and then soaked in liquid nitrogen. Seeds and root systems were lyophilized and then scratched in order to extract DNA from the seed surface and root adhering soil. DNA extractions from 500 mg of rhizosphere soils or seeds teguments were performed using a FastDNA™ SPIN Kit for Soil kit (MP Biomedicals, USA). DNA was also extracted from 1 ml of inoculum solution in order to ascertain the cell concentration of the inoculants. The seed and root bacterial colonization was assessed by real-time PCR as described by Von Felten et al. (2010). Real-time PCR were performed using the LightCycler® FastStart DNA Master SYBR® Green I kit and a LC-480 LightCycler (Roche Applied Science, Indianapolis, IN). 5 ng of extracted DNA were used to assess the abundance of seed and root colonization by the F113 parental and mutant strains using F113_1_for and F113_1_rev primers with a detection limit of 10^4 cells.seed^{-1} and 10^4 cells.g^{-1} of rhizosphere (Von Felten et al. 2010). Real-time PCR quantification data were converted to gene copy number per gram of lyophilized root as described by Couillerot et al. (2012).

Statistical analysis

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Regarding assays on each plant-beneficial function, data were log-transformed in order to respect normal distribution and variances homogeneity and a one-way ANalysis Of VAriance and Tukey's HSD tests were performed to detect significant differences between strains. *Arabidopsis* root hair length data were compared using Kruskal-Wallis tests followed by post-hoc non-parametric multiple comparisons. *Arabidopsis* primary root length data were square-root transformed in order to respect variance homogeneity and normality and a one-way ANOVA coupled with Tukey's HSD tests was performed. Ethylene emission data were expressed related to non-inoculated condition and compared using ANOVA coupled with Tukey's HSD tests. The comparison of root colonization between strains at the same sampling time was analyzed by ANOVA and Tukey's HSD tests. The comparison of root colonization across the time for each strain was analyzed by ANOVA and Fisher's LSD tests. The effects of strain inoculation on maize growth parameters were compared using ANOVA and Tukey's HSD tests and represented using star plot representation coupled to hierarchical clustering analysis performed on the raw data (distance model: Euclidean; agglomeration method used: UPGMA). All data were presented as mean ± standard error. All analyses were performed at $P < 0.05$, using R studio software (RStudio Team, 2015).

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FIG. LEGENDS

Fig.1: Effect of the deletion of F113 plant beneficial functions (PBFs) on each PBF activity of the strain. A: 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity; B: Phosphate solubilization assay; C: N_2O quantification; D: 2,4-diacetylphloroglucinol (DAPG) quantification. ND: non-detected. Statistical differences between strains for each tested PBF are indicated with letter a-c. (ANOVA and Tukey's HSD test, $P < 0.05$). Absence of letter indicates that differences were not significant.

Fig. 2: Growth curve of the *P. kilonensis* F113 and its PBF mutants in King's B broth at 28°C. The data represent the means of five replicate cultures (standard errors were not presented but lower than 10%). The experiment was done three times independently.

Fig. 3: Comparison of maize root colonization levels between *P. kilonensis* F113 and its PBF mutants. The grey color corresponds to root colonization at the moment of the seed inoculation (day 0), dark at 8 dpi and white at 20 dpi. Statistical differences across the time are represented with letters A-C. Statistical differences between strains at a given time are represented with letters a-c. The dotted line corresponds to the qPCR detection limit. NI: Non-inoculated condition.

Fig. 4: Comparison of the effects of *P. kilonensis* F113 PBF mutants on maize growth by hierarchical clustering. For each plant parameters, ratio between data obtained with mutants and with F113 were calculated and expressed in percent. Root and shoot measured parameters are colored respectively in brown and green on the star plot. LS#1: surface of the leaf number 1 (same nomenclature for leaf 2 and 3); LSTot: Total leaf surface; Chl: Chlorophyll content in leaf number 3; FSM: Fresh shoot mass; DSM: Dry shoot mass. L: total root length, S: Total root surface; D: Root diameter; V: root volume; T: Root tips; FRM: Fresh root mass; DRM: Dry root mass. R/S represents the ratio between the dry root biomass and the dry shoot biomass. Raw data were processed through hierarchical clustering analysis using Euclidean model and UPGMA as agglomeration method.

Fig. 5: Effects of the inoculation of *P. kilonensis* F113 and its PBF mutants on the length of *Arabidopsis* primary roots (A) and root hairs (B). NI: Non-inoculated condition. Conditions harboring the same letter were not significantly different (ANOVA, Tukey's HSD test, $P < 0.05$).

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Fig. 6: Plant ethylene emission in response to the inoculation of *Arabidopsis* plantlets with *P. kilonensis* F113 and its PBF mutants. Plant ethylene emission was calculated related to the plant ethylene emission for the non-inoculated condition (NI). Conditions harboring the same letter were not significantly different (ANOVA, Tukey's HSD test, $P < 0.05$).

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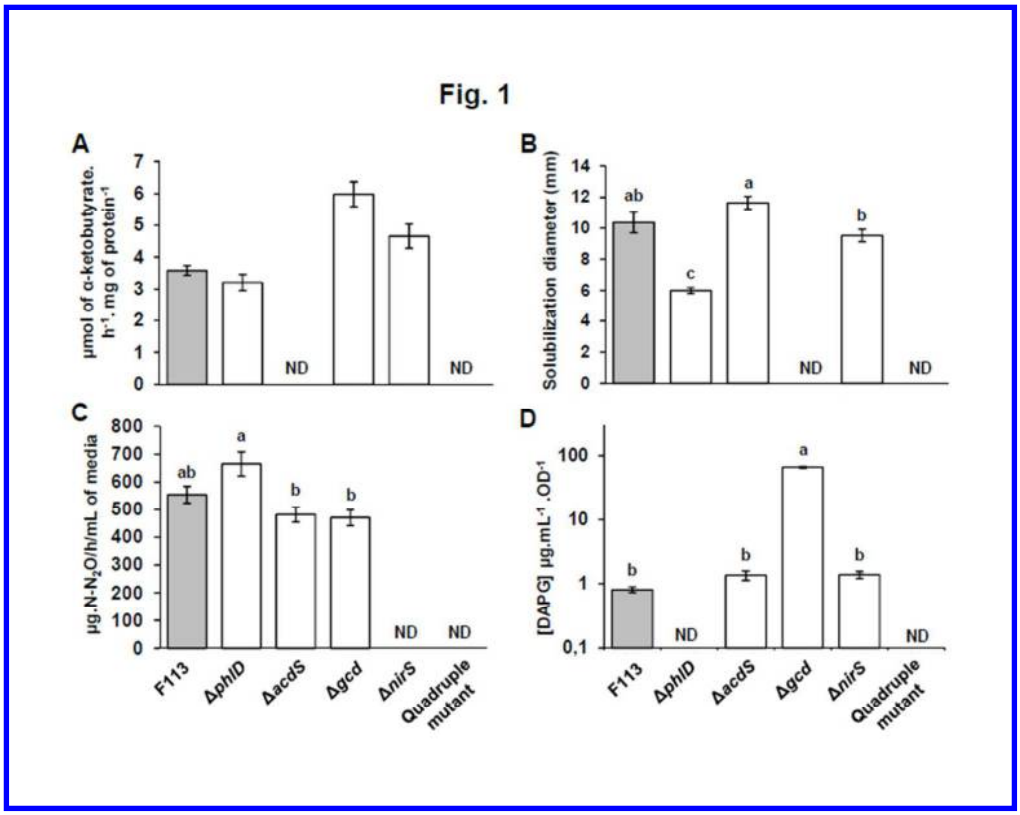

Fig. 1

281x222mm (72 x 72 DPI)

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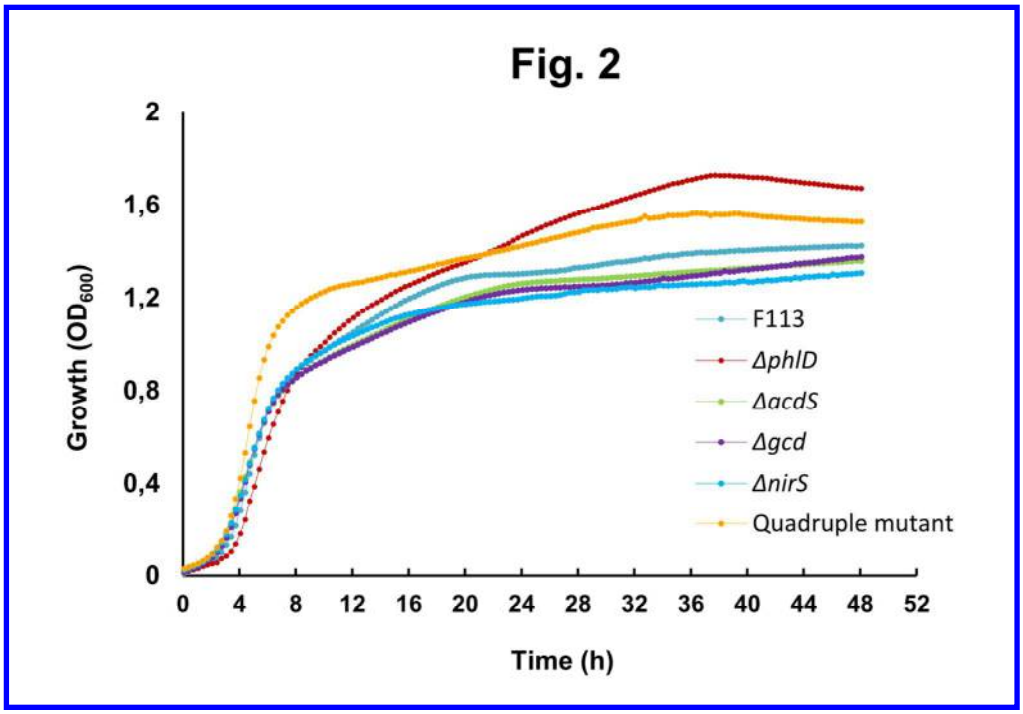

Fig. 2

132x91mm (300 x 300 DPI)

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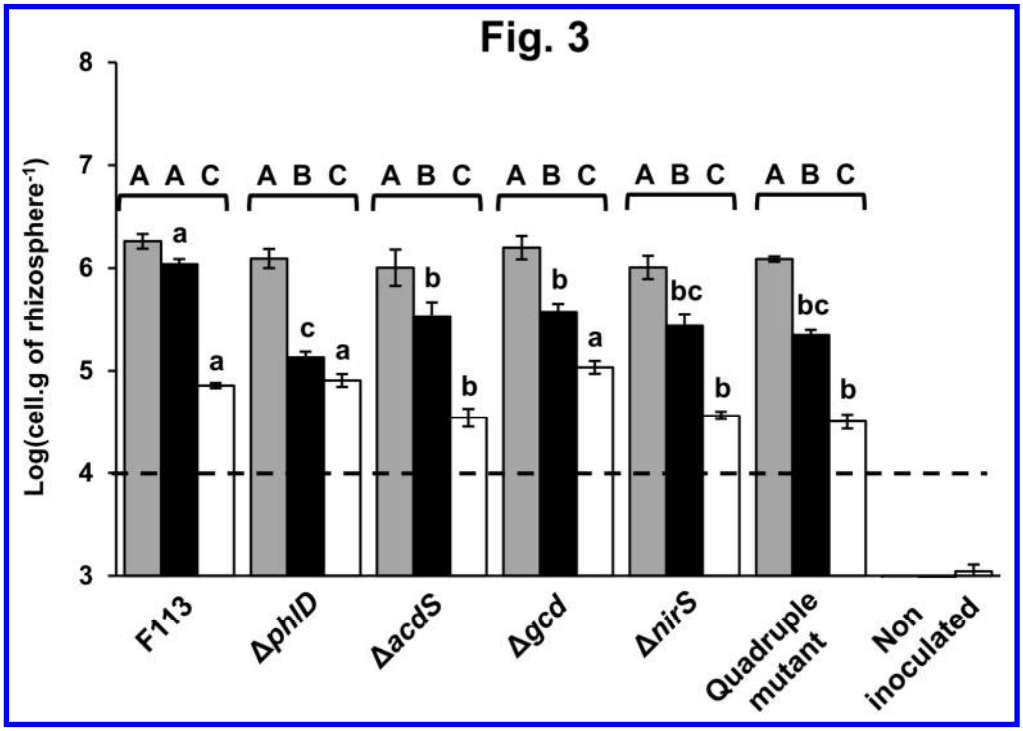

Fig. 3

133x94mm (300 x 300 DPI)

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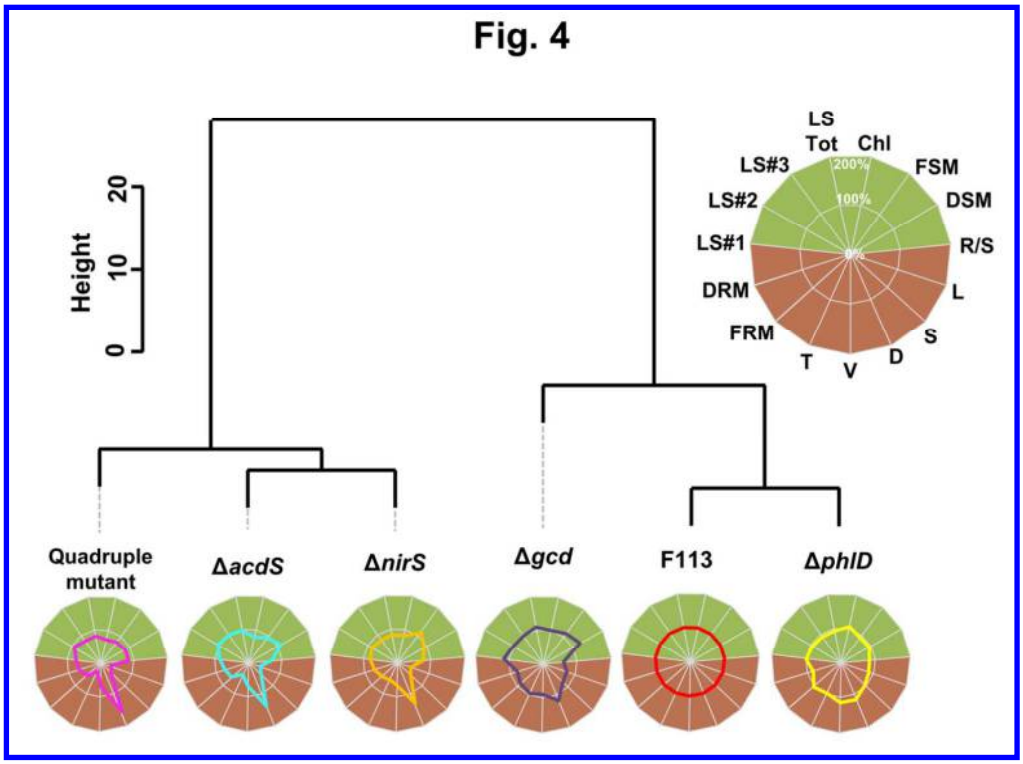

Fig. 4

112x83mm (300 x 300 DPI)

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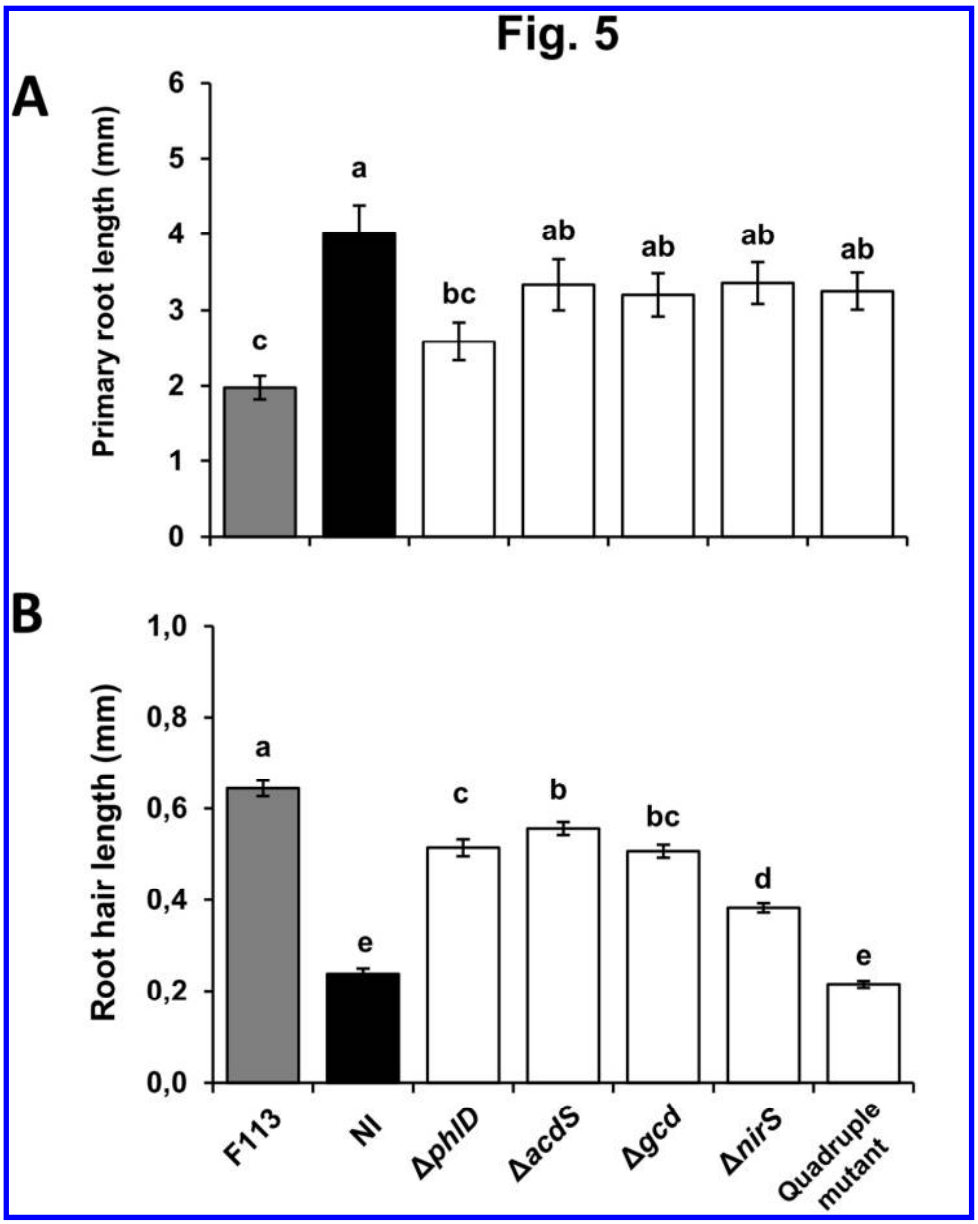

Fig. 5

113x142mm (300 x 300 DPI)

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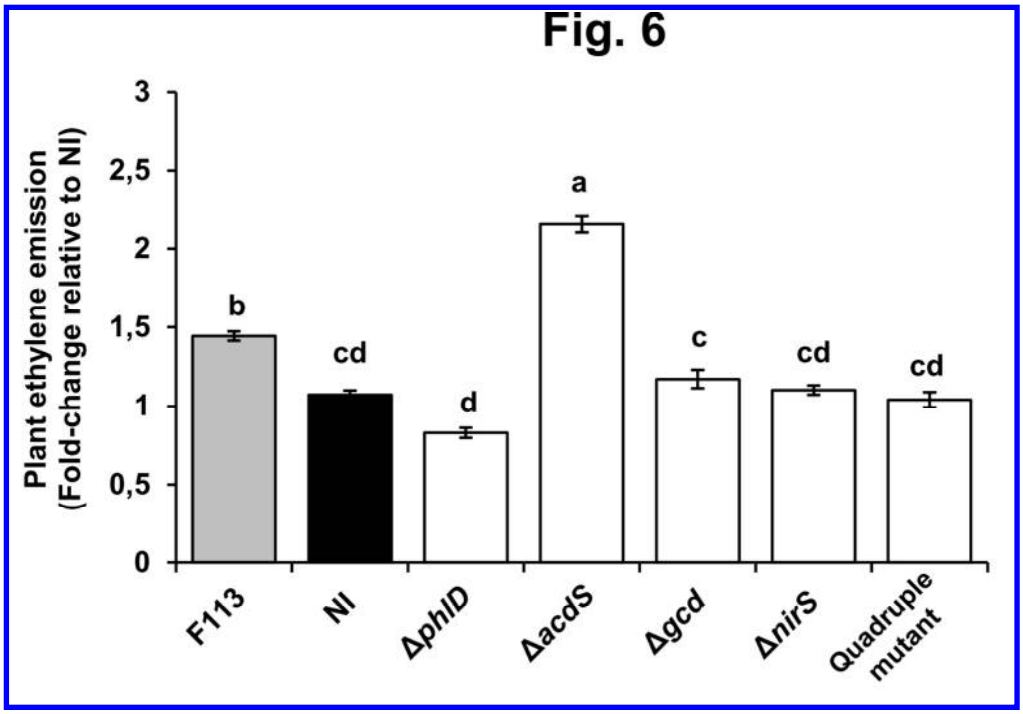

Fig. 6

118x81mm (300 x 300 DPI)

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Supplementary data

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Molecular Plant-Microbe Interactions

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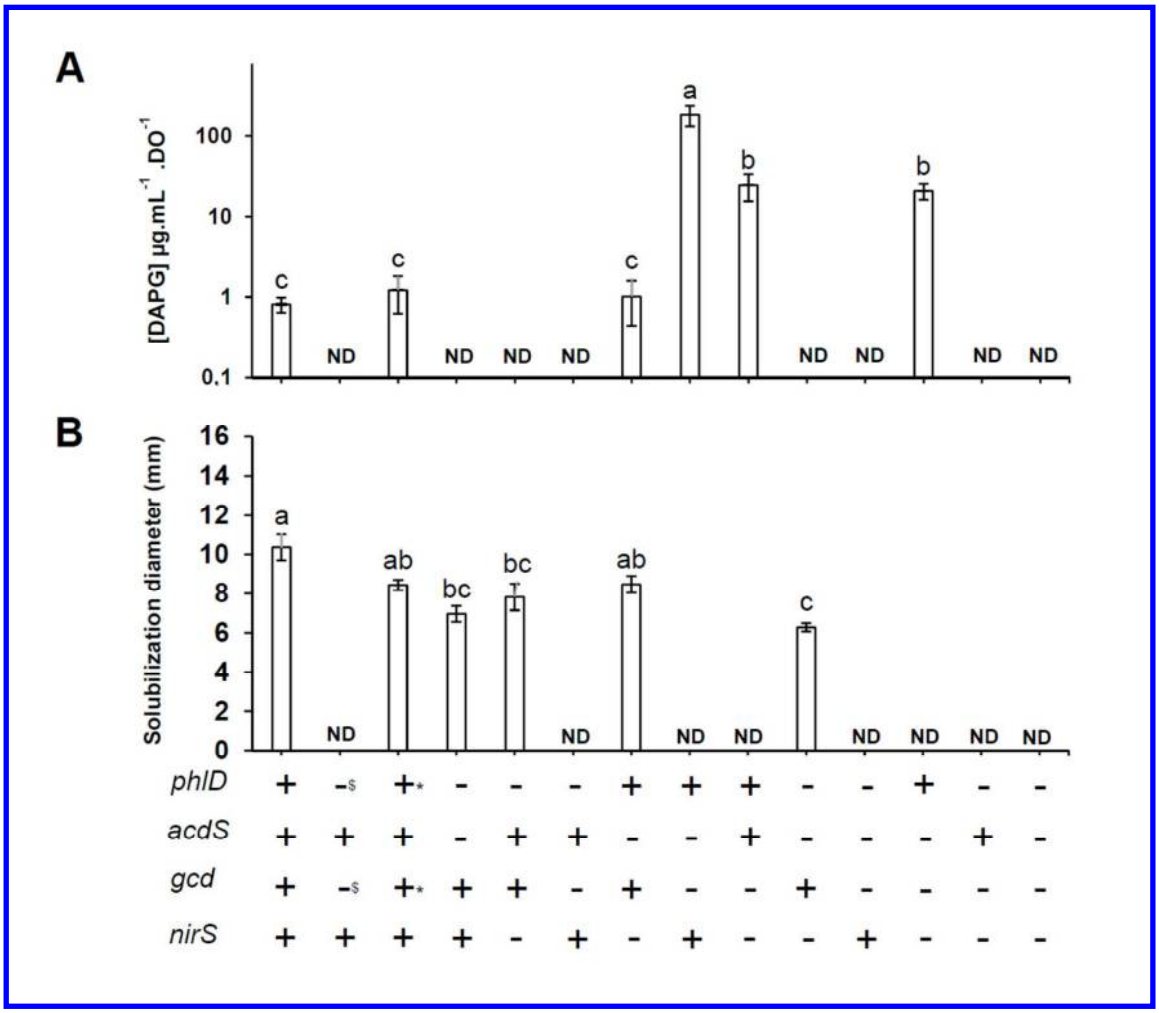

Fig. S1: Effect of the deletion (simple, double, triple, quadruple) of F113 plant beneficial functions (PBFs) on the production of 2,4-diacetylphloroglucinol (A) and phosphate solubilization activities (B). ND: non-detected. Statistical differences between strains for each tested PBF are indicated with letter a-c. Error bars correspond to standard error. (ANOVA and Tukey's HSD test, $P < 0.05$). $^{\$}$: results obtained for the 2 simple mutants Δ*phlD* or Δ*gcD* (reminder from Fig. 1); *: results obtained for the complemented Δ*phlD* and Δ*gcD* mutants.

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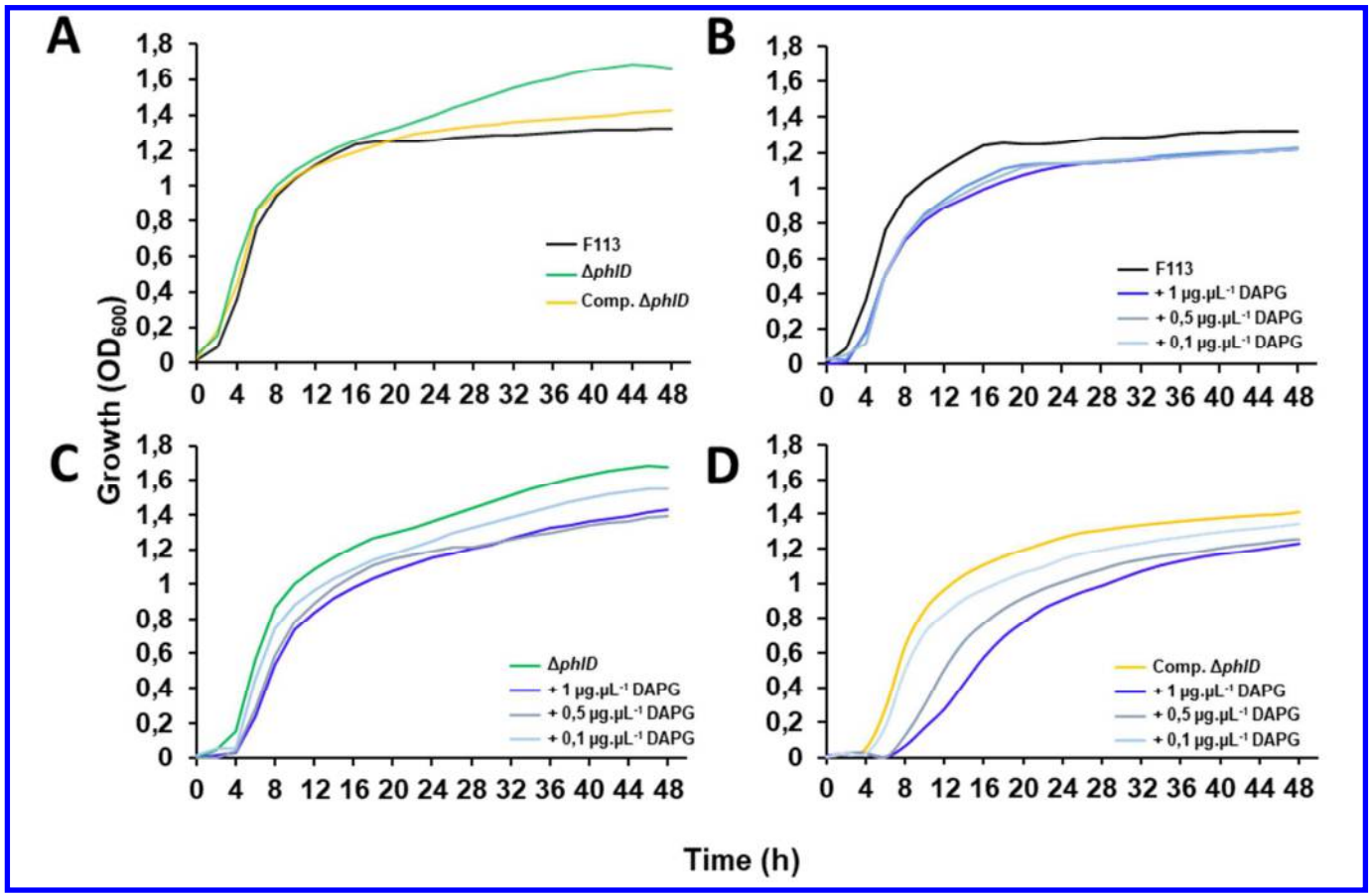

Fig. S2: Effect of the addition of DAPG on growth curves of the *P. fluorescens* wild-type F113, the Δ*phlD* mutant, and the Δ*phlD* complemented mutant with restored DAPG production.

Growth curves of F113 WT, Δ*phlD* and complemented Δ*phlD* (**A**). Effect of increasing DAPG concentrations on F113 WT (**B**), the Δ*phlD* mutant (**C**) and the complemented Δ*phlD* mutant (**D**). Comp.: Complemented. Results are presented as means of five replicate cultures (standard deviations were not presented but lower than 10%). The experiment was done three times independently.

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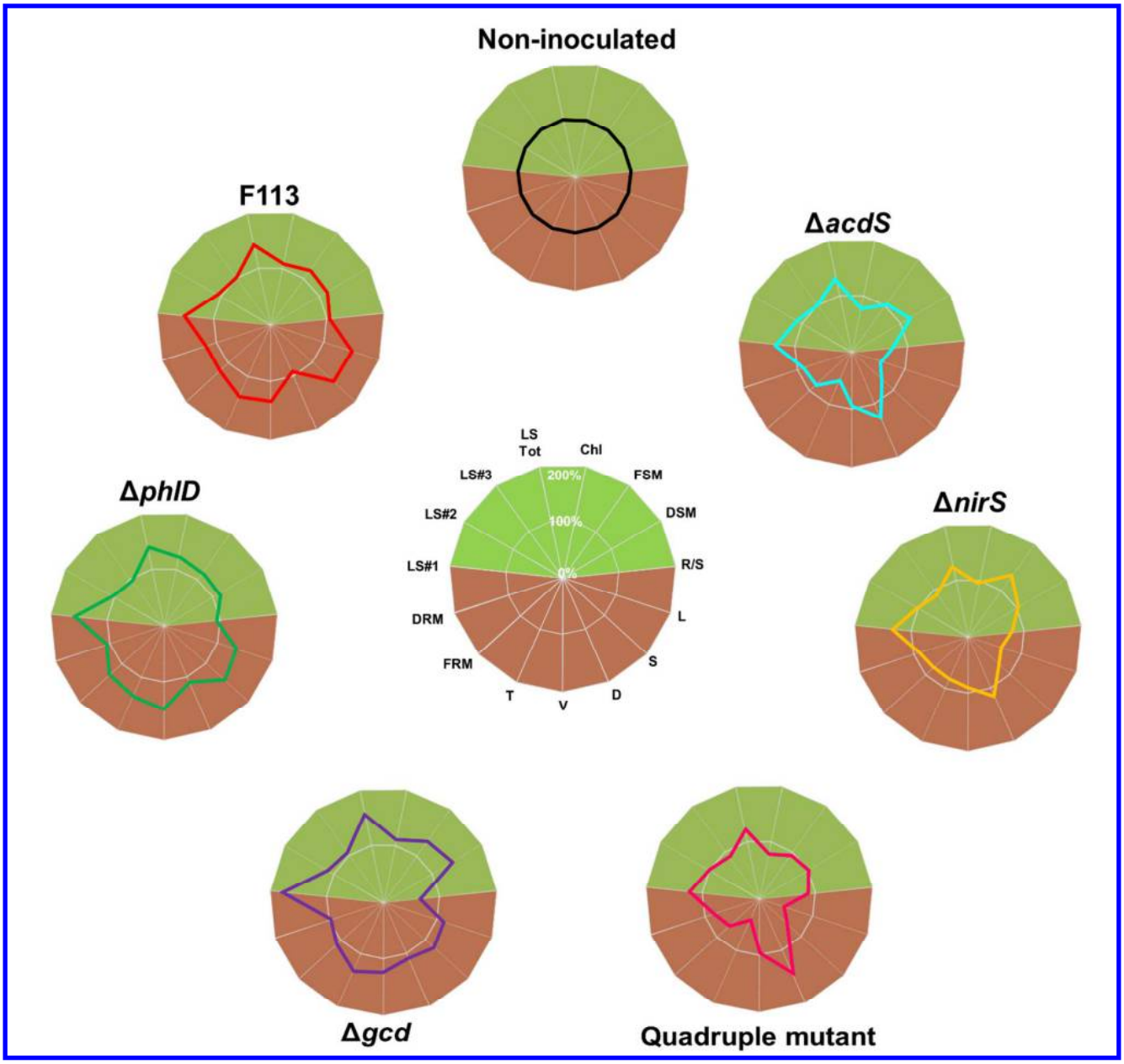

Fig. S3: Effects of *P. fluorescens* F113 and its PBF mutants on maize growth.
For each plant parameters, ratio between data obtained with mutants and with F113 were calculated and expressed in percent. Root and shoot measured parameters are colored respectively in brown and green on the star plot. **LS#1**: surface of the leaf number 1 (same nomenclature for leaf 2 and 3); **LSTot**: Total leaf surface; **Chl**: chlorophyll content in leaf number 3 measured with a chlorophyll content meter (Hansatech instruments model CL-01; Dinç et al. 2012); **FSM**: Fresh shoot mass; **DSM**: Dry shoot mass. L: total root length, **S**: Total root surface; **D**: Root diameter; **V**: root volume; **T**: Root tips; **FRM**: Fresh root mass; **DRM**: Dry root mass. **R/S** ratio represents the ratio between the dry root biomass and the dry shoot biomass.

Dinç, E., Ceppi, M.G., Tóth, S.Z., Bottka, S., Schansker, G. 2012. The chl a fluorescence intensity is remarkably insensitive to changes in the chlorophyll content of the leaf as long as the chl a/b ratio remains unaffected. Biochim. Biophys. Acta 1817:770-779.

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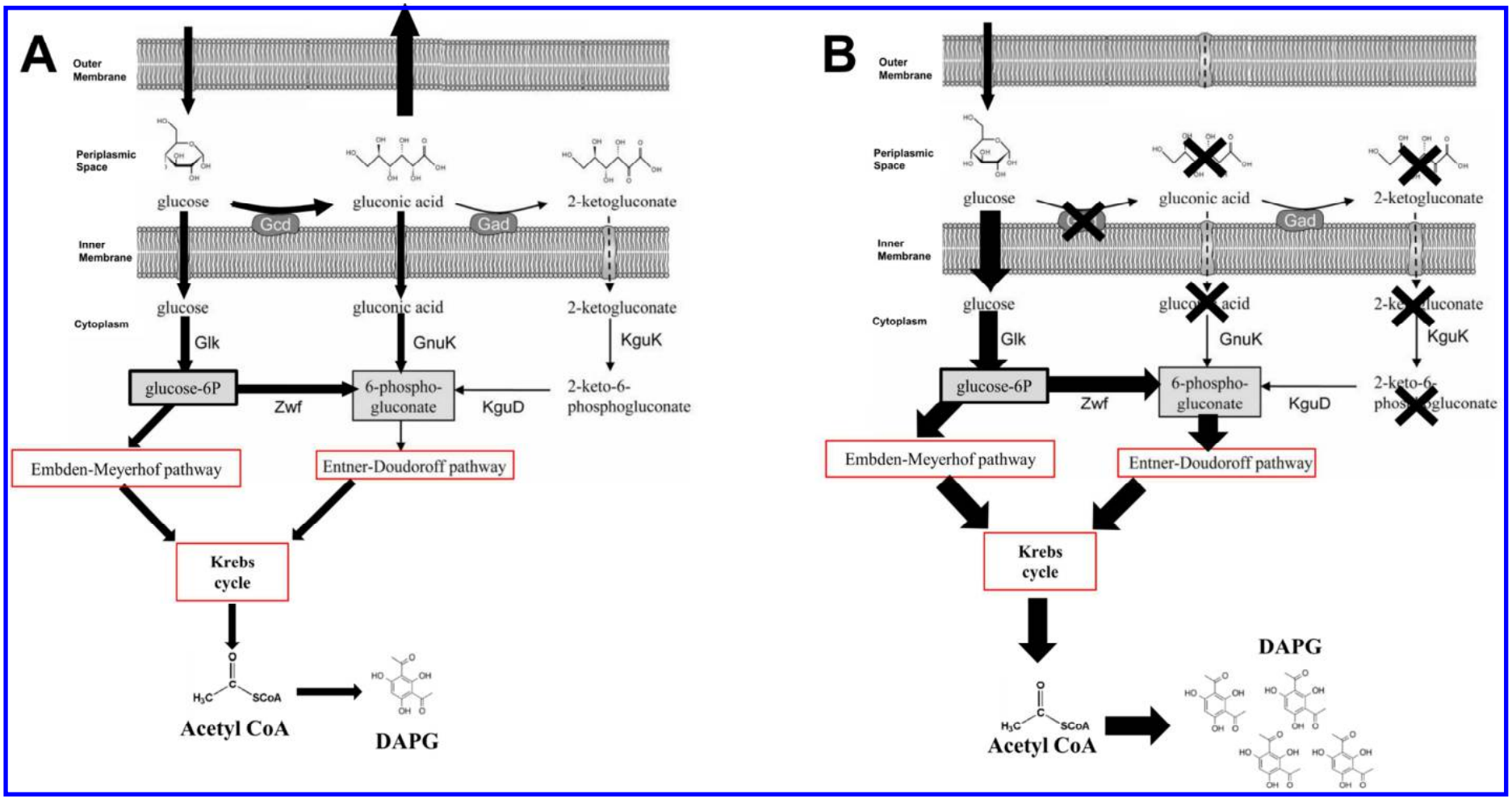

Fig. S4: Model presenting potential metabolic regulation involved in DAPG production in *P. fluorescens* F113.
Glucose catabolism in F113 (A) and F113 *gcd* mutant (B). Enzymes involved in glucose metabolism are abbreviated using the same nomenclature as de Werra *et al.* (2009). The corresponding enzyme functions and F113 gene names are indicated in parenthesis: Gcd (glucose dehydrogenase - *gcd*); Glk (glucokinase - *PSF113_1279*), Gad (Gluconate dehydrogenase - *PSF113_4842*); Zwf (glucose-6-phosphate-1-dehydrogenase - *PSF113_1290* and *PSF113_2674*); GnuK (gluconokinase - *PSF113_1311*); KguD (2-ketogluconate 6-phosphate reductase - *kguK*); KguK (2-ketogluconate kinase – *kguK*).

de Werra, P., Péchy-Tarr, M., Keel, C., Maurhofer, M. 2009. Role of gluconic acid production in the regulation of biocontrol traits of *Pseudomonas fluorescens* CHA0. Appl. Environ. Microbiol. 75:4162–4174.

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Table S1: Slope comparison at the exponential growth stage and OD_{600} at the stationary phase for F113 WT and its PBF mutants,

Strains	Growth curve slopes at exponential growth stage	OD_{600} at the stationary phase
F113 WT	0.20 ± 0.02 b	1.29 ± 0.06 b
Δ*phlD*	0.20 ± 0.02 b	1.54 ± 0.05 a
Δ*acdS*	0.17 ± 0.04 b	1.29 ± 0.09 b
Δ*gcd*	0.18 ± 0.03 b	1.37 ± 0.08 b
Δ*nirS*	0.18 ± 0.03 b	1.35 ± 0.12 b
Quadruple mutant	0.25 ± 0.02 a	1.52 ± 0.01 a

Data were presented as mean ± standard deviation. Letters indicate statistical relations between strains for each tested PBF activities (ANOVA and Tukey's HSD test, $P < 0.05$)

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Table S2: Effect of *P. fluorescens* F113 and its related mutants on maize root architecture

	Total root length (cm)	Total root surface (cm^2)	Root diameter (mm)	Root total volume (cm^3)	Number of root tips	Fresh root weight (g)	Dry root weight (g)
F113	301.28 ± 61.68 a	70.96 ± 11.68 a	0.81 ± 0.05 b	1.35 ± 0.17 ab	2636.00 ± 755.92 a	1.39 ± 0.14 a	0.18 ± 0.04 a
Δ*phlD*	262.55 ± 43.59 a	68.59 ± 7.40 a	0.92 ± 0.09 ab	1.50 ± 0.13 a	2535.75 ± 551.14 a	1.44 ± 0.12 a	0.15 ± 0.01 ab
Δ*acdS*	108.83 ± 18.92 bc	35.47 ± 3.97 bc	1.12 ± 0.09 ab	0.96 ± 0.08 ab	995.29 ± 235.46 b	0.95 ± 0.09 b	0.13 ± 0.03 ab
Δ*gcd*	223.32 ± 61.34 b	56.39 ± 9.04 bc	0.93 ± 0.10 ab	1.23 ± 0.12 ab	2486.00 ± 592.98 a	1.24 ± 0.11 ab	0.14 ± 0.02 ab
Δ*nirS*	123.30 ± 27.81 bc	35.71 ± 5.44 bc	1.02 ± 0.08 ab	0.86 ± 0.10 b	1455.38 ± 400.02 b	0.86 ± 0.13 b	0.12 ± 0.03 b
ΔΔΔΔ	86.85 ± 17.69 c	30.81 ± 4.35 c	1.26 ± 0.13 a	0.94 ± 0.16 ab	760.13 ± 163.47 b	0.80 ± 0.06 b	0.12 ± 0.02 b
NI	201.23 ± 51.83 bc	48.66 ± 8.85 bc	0.90 ± 0.11 ab	1.01 ± 0.14 ab	1918.63 ± 506.78 a	1.14 ± 0.12 ab	0.14 ± 0.04 ab

* AU: Arbitrary Unit. Different letters correspond to significant differences for each plant parameter (ANOVA coupled with HSD's Tukey test *P*=0.05).

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Table S3: Effect of *P. fluorescens* F113 and its related mutants on maize aerial parts

	Leaf surface 1 (cm²)	Leaf surface 2 (cm²)	Leaf surface 3 (cm²)	Total Leaf surface (cm²)	Chlorophyll content (AU*)	Fresh shoot weight (g)	Dry shoot weight (g)	R/S ratio
F113	22.91 ± 2.50 a	14.22 ± 0.63 a	6.39 ± 0.33 a	43.52 ± 3.18 a	9.03 ± 0.95 ab	1.31 ± 0.15 ab	0.14 ± 0.02 ab	1.30 ± 0.17 ab
Δ*phlD*	23.33 ± 1.41 a	13.97 ± 0.33 a	6.15 ± 0.14 a	43.45 ± 1.68 a	10.15 ± 0.78 a	1.26 ± 0.08 ab	0.14 ± 0.01 ab	1.11 ± 0.08 ab
Δ*acdS*	20.33 ± 2.04 ab	14.90 ± 0.75 a	6.46 ± 0.51 a	41.69 ± 3.01 ab	6.74 ± 0.39 b	1.22 ± 0.13 ab	0.16 ± 0.02 ab	0.92 ± 0.17 ab
Δ*gcd*	26.29 ± 2.00 a	14.54 ± 0.36 a	6.50 ± 0.21 a	47.33 ± 2.37 a	9.19 ± 0.80 ab	1.47 ± 0.12 ab	0.18 ± 0.01 a	0.81 ± 0.05 b
Δ*nirS*	18.91 ± 1.56 ab	12.84 ± 0.55 a	5.44 ± 0.32 a	37.20 ± 2.17 ab	7.69 ± 0.49 ab	1.52 ± 0.21 a	0.13 ± 0.01 ab	0.96 ± 0.09 ab
ΔΔΔΔ	18.16 ± 2.14 ab	13.43 ± 0.80 a	5.54 ± 0.32 a	37.13 ± 3.21 ab	6.49 ± 0.51 b	1.05 ± 0.11 ab	0.12 ± 0.01 ab	1.06 ± 0.08 ab
NI	14.76 ± 1.56 b	13.12 ± 0.85 a	6.12 ± 0.33 a	30.31 ± 2.39 b	8.27 ± 0.67 ab	1.12 ± 0.18 b	0.13 ± 0.02 b	1.26 ± 0.15 a

* AU: Arbitrary Unit. Different letters correspond to significant differences for each plant parameter (ANOVA coupled with HSD's Tukey test P=0.05).

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Table S4: Bacterial strains and plasmids used in this study

Bacterial strain or plasmid	Relevant Characteristics[a]	Source or reference
Bacterial Strains		
Pseudomonas fluorescens		
F113	Wild-type, Phl$^+$, *acdS*$^+$, *gcd*$^+$, *nirS*$^+$ HCN$^+$	Shanahan et al. 1992
Δ*phlD*	F113 strain *phlD* gene deleted	This study
Δ*acdS*	F113 strain *acdS* gene deleted	This study
Δ*gcd*	F113 strain *gcd* gene deleted	This study
Δ*nirS*	F113 strain *nirS* gene deleted	This study
Δ*phlD* Δ*acdS* Δ*gcd* Δ*nirS*	F113 quadruple mutant	This study
Δ*phlD*Comp	Δ*phlD* with restored DAPG production capacity	This study
Escherichia coli		
JM109	Host strain for the plasmids transformation	Promega
Plasmids		
pGEM-T easy vector	Cloning vector; Amp^r;	Promega
pBBR1-MCS5	Cloning vector used for complementation, Gm^r	Kovach et al. 1995
pCM184	Cloning vector used for homologous recombination, Amp^r, Km^r, Tc^r	Marx and Lidstrom 2002
pCM157	Containing Cre recombinase activity, Amp^r, Tc^r	Marx and Lidstrom 2002
pGEM-T easy:*phlD*	*phlD* cloned into pGEM-T easy, Amp^r	This study
pGEM-T easy:UP*phlD*	Upstream sequence of *phlD* cloned into pGEM-T easy, Amp^r	This study
pGEM-T easy:DW*phlD*	Downstream sequence of *phlD* cloned into pGEM-T easy, Amp^r	This study
pGEM-T easy:UP*acdS*	Upstream sequence of *acdS* cloned into pGEM-T easy, Amp^r	This study
pGEM-T easy:DW*acdS*	Downstream sequence of *acdS* cloned into pGEM-T easy, Amp^r	This study
pGEM-T easy:UP*gcd*	Upstream sequence of *gcd* cloned into pGEM-T easy, Amp^r	This study
pGEM-T easy:DW*gcd*	Downstream sequence of *gcd* cloned into pGEM-T easy, Amp^r	This study
pGEM-T easy:UP*nirS*	Upstream sequence of *nirS* cloned into pGEM-T easy, Amp^r	This study
pGEM-T easy:DW*nirS*	Downstream sequence of *nirS* cloned into pGEM-T easy, Amp^r	This study
pCM184:*phlD*	Upstream and downstream sequence of *phlD* into pCM184, Amp^r, Km^r, Tc^r	This study
pCM184:*acdS*	Upstream and downstream sequence of *acdS* into pCM184, Amp^r, Km^r, Tc^r	This study
pCM184:*gcd*	Upstream and downstream sequence of *gcd* into pCM184, Amp^r, Km^r, Tc^r	This study
pCM184:*nirS*	Upstream and downstream sequence of *nirS* into pCM184, Amp^r, Km^r, Tc^r	This study
pBBR1-MCS5:*phlD*	*phlD* cloned into pBBR1-MCS5, Gm^r	This study

[a]: Amp^r Ampicillin resistant; Km^r Kanamycin resistant; Tc^r Tetracycline resistant, Gm^r, Gentamycin resistant

Kovach, M.E., Elzer, P.H., Hill, D.S. et al. 1995. Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. Gene 166: 175–176; Marx, C.J., Lidstrom, M.E. 2002. Broad-host-range *cre-lox* system for antibiotic marker recycling in gram-negative bacteria. Biotechniques 33:1062–1067; Shanahan, P., O'Sullivan, D.J., Simpson, P., Glennon, J.D., O'Gara, F. 1992. Isolation of 2,4-diacetylphloroglucinol from a fluorescent Pseudomonad and investigation of physiological parameters influencing its production. Appl. Environ. Microbiol. 58:353–358.

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Table S5: Primers used in this study

Targeted regions	Names of primers	Sequences	Sizes of amplification	Reference
Insertion verification into pGEMTeasy	M13 F	GTTTTCCCAGTCACGAC	Variable according to the cloned gene	Promega
	M13 R	CAGGAAACAGCTATGAC		
Insertion verification into pBBR1-MCS5	pBBR1MCS5_F1	TGCAAGGCGATTAAGTTGGGTA	Variable according to the cloned gene	Kovach et al. 1995
	pBBR1MCS5_R1	TATGACCATGATTACGCCAAGC		
phlD gene amplification	phlDCompF1	ACTTTATTGGCTTCTCGCCG	1104pb	This study
	phlDCompR1	GGGTGTCGGTGTTTTATCGG		This study
pCM184 insertion site 1	S1F	ACGGAAATGTTGAATACTCATACTC	Variable according to the cloned gene	This study
	S1R	CGTTTCCCGTTGAATATGGC		This study
pCM184 insertion site 2	S2F	GCAGGTCCTAGATAACTTCG	Variable according to the cloned gene	This study
	S2R	GGCTGGATCCTCTAGTGAG		This study
Upstream region of *phlD*	phlDF1	TAGAGGTACCAGGCCACTAA	700pb	This study
	phlDR1	†CAATTGTGACAGACCGATAAAACACCG		This study
Downstream region of *phlD*	phlDF2	CATGACAAGTCCTCGGCGAGA	753pb	This study
	phlDR2	GGGATTTTGCACATTACTGA		This study
Upstream region of *acdS*	acdSF1	TAAGCGTAGAACTGCTTTTGG	773pb	This study
	acdSR1	‡CAGCTGTAGAACGACAGTCGGAAGAT		This study
Downstream region of *acdS*	acdSF2	ATTCAGTCGTGTCAGGTTTT	724pb	This study
	acdSR2	CATGACTCTGCTCCTTGATAT		This study
Upstream region of *gcd*	gcdF1	TTTCATCGATTTCGACTACG	793pb	This study
	gcdR1	†CAATTGTAAGCCCAAGCTGCGCTC		This study
Downstream region of *gcd*	gcdF2	CATAGTGTGTTTCTCCGTGAC	750pb	This study
	gcdR2	CTTATCAATCCCCATGACTC		This study
Upstream region of *nirS*	nirSF1	‡CAGCTGCAGCAGGTGTTGCAGTTG	711pb	This study
	nirSR1	†CAATTGAAGAACGTGATCAAGGACAAG		This study
Downstream region of *nirS*	nirSF2	TTTTTTGTGGCTGATCAGCAT	702pb	This study
	nirSR2	CTTCATCCCCTTGAGCAG		This study
External primer	phlDExtf	ATTGAAGGAACAGTAGCCAG	2378/1334pb *	This study
	phlDExtr	TGCAAAGGATATCGATGTGG		This study
External primer	acdSExtf	ACAGCGTGAGCATTAGACAA	2896/1885pb	This study
	acdSExtr	ACTCTTCAAGTTCACCGCAG		This study
External primer	gcdExtf	CCATGAACCGTGAAGGTATT	4357/1949pb	This study
	gcdExtr	GTCAGTACCGTGAAGAAACT		This study
External primer	nirSExtf	AGGAGAGGAAAGAACGGAT	3550/1972pb	This study
	nirSExtr	TTCAGCCAAAGTGGACATC		This study

† : MunI (MfeI) restriction site ; ‡ : PvuII restriction site ; *: size in F113 WT / size in the deleted mutant

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